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(54) Title: METHOD FOR THE SYNTHESIS OF POLY-PYRROLE AND POLY-IMIDAZOLE CARBOXAMIDES ON A SOLID SUPPORT

(57) Abstract

The present invention describes a novel method for the solid phase synthesis of polyamides containing imidazole and pyrrole carboxamides. The polyamides are prepared on a solid support from aromatic carboxylic acids and aromatic amines with high stepwise coupling yields (> 99 %), providing milligram quantities of highly pure polyamides. The present invention also describes the synthesis of analogs of the natural products Netropsin and Distamycin A, two antiviral antibiotics. The present invention also describes a novel method for the solid phase synthesis of imidazole and pyrrole carboxamide polyamide-oligonucleotide conjugates. This methodology will greatly increase both the complexity and quantity of minor-groove binding polyamides and minor-groove binding polyamide-oligonucleotide conjugates which can be synthesized and tested.

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METHOD FOR THE SYNTHESIS OF POLY-PYRROLE AND POLY-IMIDAZOLE CARBOXAMIDES ON A SOLID SUPPORT

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FIELD OF THE INVENTION

This invention relates to the field of peptide chemistry. Specifically, this invention relates to a novel process for preparing polyamides and polyamide conjugates containing imidazole and pyrrole carboxamides using solid state chemistry. Also included in this invention is a simple and effective method for preparing analogs of the antiviral antibiotics Netropsin and Distamycin A.

BACKGROUND OF THE INVENTION

Proteins and peptides play a critical role in virtually all biological processes, functioning as enzymes, hormones, antibodies, growth factors, ion carriers, antibiotics, toxins, and neuropeptides. Biologically active proteins and peptides, therefore, have been a major target for chemical synthesis. Chemical synthesis is used to verify structure and to study the relationship between structure and function, with the goal of designing novel compounds for potential therapeutic use. Thus, modified or novel peptides may be synthesized which have improved therapeutic activity and/or reduced side effects.

There are two basic methods for synthesizing proteins and peptides: the chemistry is either carried out in solution (solution phase) or on a solid support (solid phase). A major disadvantage of solution phase synthesis

of peptides is the poor solubility of the protected peptide intermediates in organic solvents. Additionally, solution phase synthesis requires extensive experience on the part of the scientist and the purifications are difficult and time consuming. Solid phase synthesis overcomes these problems and thus, has become the method of choice in synthesizing peptides and proteins.

The basic approach for solid phase peptide synthesis is illustrated in Figure 1. Briefly, the carboxy-terminal amino acid of the peptide to be synthesized is protected and covalently attached to a solid support, typically a resin. The subsequent amino acids (which have also been protected) are then sequentially added. When the synthesis is complete the peptide is deprotected, cleaved from the resin and purified. Because the molecules being synthesized are so large it is imperative that the steps proceed rapidly, in high yields and with minimal side reactions.

The most commonly used solid supports are cross-linked polystyrene and polydimethylacrylamide resins, which are both derivatives of polyethylene. In 1978, Merrifield and coworkers introduced the tert-butyloxycarbonylaminoacyl-4-(oxymethyl)phenyl-acetamidomethyl-resin (PAM resin). a novel polystyrene resin for solid phase peptide synthesis (Mitchell *et al.* (1978) J. Org. Chem. 43:2845-2852). PAM resin has a preformed resin ester linkage which is stable to trifluoroacetic acid and can be cleaved under a variety of conditions including, liquid hydrogen fluoride. aminolysis. hydrolysis, hydrazinolysis, catalytic hydrogenation, or lithium borohydride to give a peptide acid, amide, hydrazide. or primary alcohol (Stewart and Young (1984) in Solid Phase Peptide Synthesis, sec. ed., Pierce Chemical Company, Illinois pp. 88-95).

Netropsin and Distamycin A (Figures 2A and 2B) are heterocyclic polyamides, containing imidazole (Im) and pyrrole (Py) carboxamides. These compounds are isolated from *Streptomyces distallicus* and exhibit antibiotic, antiviral and antitumor activity. Other members of this family of antibiotics include noformycin (Diana (1973) J. Med. Chem. 16:3774-3779), kikumycin B (Takaishi *et al.* (1972) Tetrahedron Lett. 1873), and anthelvencin (Probst *et al.* (1965) Antimicrob. Agents Chemother. 789). Netropsin and Distamycin A are two examples of the many small molecules (MW < 2 kD) which can bind and/or cleave DNA with modest sequence specificity (Krugh (1994) Curr. Opin. Struct. 4:351-364). These drugs block template function by binding to specific nucleotides in the minor groove of double-stranded DNA.

Due to the pharmaceutical potential of this family of peptides a considerable amount of research has been devoted to the study of these compounds and their analogues. The x-ray crystal structure of a 1:1 complex of Netropsin with the B-DNA dodecamer 5'-CGCGAATTCGCG-3' (SEQ ID NO:1) provides an understanding of how the sequence specificity is achieved, revealing that the amide hydrogens of the *N*-methylpyrrolecarboxamides form bifurcated hydrogen bonds with adenine N3 and thymidine O2 atoms on the floor of the minor groove. (Koopka *et al.* (1985) Proc. Natl. Acad. Sci. 82:1376; Koopka *et al.* (1985) J. Mol. Biol. 183:553). The pyrrole rings completely fill the groove excluding the guanine amino group of a G, C base pair while making extensive van der Waals contacts with the walls of the groove, thereby affording specificity for A,T sequences. (Taylor *et al.* (1985) Tetrahedron 40:457; Schultz and Dervan (1984) J. Biomol. Struct. Dyn. 1:1133). Efforts to design ligands specific for G, C containing sequences, were

largely unsuccessful (*see* e.g., Lown *et al.* (1986) Biochemistry 25:7408; Kssinger *et al.* (1987) Biochemistry 26:5590; Lee *et al.* (1987) Biochemistry 27:445; Lee *et al.* (1993) Biochemistry 32:4237), until the discovery that two polyamides combine side-by-side in the minor groove of DNA, forming a 2:1 complex with the DNA. (Pelton (1989) Proc. Natl. Acad. Sci., USA 86:5723-5727; Pelton (1990) J. Am. Chem. Soc. 112:1393-1399; Chen *et al.* (1994) M. Struct. Biol. Nat. 1:169-175; Wade *et al.* (1992) J. Am. Chem. Soc. 114:8783-8794; Mrksich *et al.* (1992) Proc. Natl. Acad. Sci., USA 89:7586-7590; Wade (1993) Biochemistry 32:11385-11389; Mrksich *et al.* (1994) J. Am. Chem. Soc. 116:7983-7988). Each ligand interacts with one of the DNA strands in the minor groove, with the imidazole nitrogen making specific hydrogen bonds with one guanine amino group. Thus, both Distamycin A and imidazole containing ligands such as the designed polyamide imidazole-pyrrole-pyrrole-dimethylaminoproplyamine (ImPyPy-Dp), 1-methylimidazole-2-carboxamide Netropsin, bind specifically in the minor groove as 2:1 polyamide/DNA complexes recognizing G, C sequences.

From studies of the 2:1 model it is now known that the combination of imidazole/pyrrole carboxamide recognize a G, C base pair, and the combination of pyrrole carboxamide/imidazole recognizes a C, G base pair, the pyrrole carboxamide/pyrrole carboxamide combination is partially degenerate for T, A and A, T. The utility of the 2:1 model as an aid in designing ligands with sequence specificity for DNA is illustrated by the designed polyamide imidazole-pyrrole-imidazole-pyrrole-dimethylaminopropylamine (ImPyImPy-Dp) which binds a four base pair core sequence 5'-GCGC-3'. This is a complete reversal of the natural specificity of Netropsin and Distamycin A.

The literature contains a number of reports of the total synthesis of various members of this family of polyamides and their analogues. All of the reported syntheses have been performed in the solution phase. The amide bond unit in these polyamides is formed from an aromatic carboxylic acid and an aromatic amine, both of which have proven problematic for solution phase coupling reactions. The aromatic acids are often unstable resulting in decarboxylation and the aromatic amines have been found to be highly air and light sensitive (Lown and Krowicki (1985) J. Org. Chem. 50:3774-3779). It was believed that the variable coupling yields, long (often > 24 hour) reaction times, numerous side products, and wide scale use of acid chloride and trichloroketone intermediates in solution phase coupling reactions would make the synthesis of the aromatic carboxamides difficult, if not impossible by solid phase methods (He *et al.* (1993) J. Am. Chem. Soc. 115:7061-7071; Church *et al.* (1990) Biochemistry 29:6827-6838; Nishiwaki *et al.* (1988) Heterocycles 27:1945-1952). Thus, to date, there have been no reported attempts to synthesize this class of compounds using solid phase methodology.

The process of developing new ligands with novel sequence specificity generally involves four stages; design, synthesis, testing, and redesign of the model (Dervan (1986) Science 232:464). While exploring the limits of the 2:1 model, the synthetic portion of the process emerged as the major limiting factor, especially when confronted with expanding the 2:1 motif to include longer sequences recognized by increasingly larger polyamides. For example, the total synthesis of hairpin octa-amides such as AcImImPy-γ-PyPyPy-G-Dp and AcPyPyPy-γ-ImImPy-G-Dp (Figures 3A and 3B) is characterized by difficult purifications. (γ represents γ-aminobutyric acid and G represents guanine.) Each polyamide would likely require more than a

months effort, even in the hands of a skilled researcher. Methods for expediting the synthesis of analogs of Distamycin A were investigated and the present invention describes a novel method for the synthesis of oligopeptides containing imidazole and pyrrole carboxamides on a solid support.

Oligonucleotide-directed triple helix formation is one of the most effective methods for accomplishing the sequence specific recognition of double helical DNA. (*See e.g.*, Moser and Dervan (1987) Science 238:645; Le Doan *et al.* (1987) Nucleic Acids Res. 15:7749; Maher *et al.* (1989) Science 245:725; Beal and Dervan (1991) Science 251:1360; Strobel *et al.* (1991) Science 254:1639; Maher *et al.* (1992) Biochemistry 31:70). Triple helices form as the result of hydrogen bonding between bases in a third strand of DNA and duplex base pairs in the double stranded DNA, via Hoogsteen base pairs. Pyrimidine rich oligonucleotides bind specifically to purine tracts in the major groove of double helical DNA parallel to the Watson-Crick (W-C) purine strand (Moser and Dervan (1987) Science 238:645). Specificity is derived from thymine (T) recognition of adenine-thymine base pairs (T-AT) base triplets and protonated cytosine (C^+) recognition of guanine-cytosine base pairs (C^+-GC). (Felsenfeld *et al.* (1957) J. Am. Chem. Soc. 79:2023; Howard *et al.* (1964) Biochem. Biophys. Res. Commun. 17:93; Rajagopal and Feigon (1989) Nature 339:637; Radhakrishnan *et al.* (1991) Biochemistry 30:9022). Purine-rich oligonucleotides, on the other hand, bind in the major groove of purine rich tracts of double helical DNA antiparallel to the W-C purine strand. (Beal and Dervan (1991) Science 251:1360). Specificity is derived from guanine recognition of GC base pairs (G-GC base triplets) and adenine recognition of AT base pairs (A-AT base triplets). (Durland *et al.* (1991) Biochemistry 30:9246; Pilch *et al.* (1991) Biochemistry 30:6081; Radhakrishnan *et al.* (1991)

J. Mol. Biol. 221:1403; Beal and Dervan (1992) Nucleic Acids Res. 20: 2773). Oligonucleotide directed triple helix formation is therefore limited mainly to purine tracts.

A major challenge in the sequence specific recognition of duplex DNA by triple helix formation is designing oligonucleotides capable of binding all four base pairs. Efforts toward this goal have included the design of non-natural heterocycles for the completion of the triplex code and the design of oligonucleotides capable of binding alternate strands of duplex DNA by triple-helix formation. (Beal and Dervan (1992) J. Am. Chem. Soc. 114:4976-4982; Stiltz and Dervan (1992) Biochem. 9:2177-2185; Koshlap *et al.* (1993) J. Am. Chem. Soc. 115:7908-7909).

An increasingly versatile method for accomplishing the sequence specific recognition of DNA is the use of natural DNA binding molecules with altered sequence specificity. (Dervan (1986) Science 232:464). The construction of oligonucleotide-minor groove polyamide conjugates, using natural DNA binding molecules, such as Netropsin and Distamycin A, offers a promising method for expanding the number of sequences which can be targeted by oligonucleotide directed triple helix formation.

A number of methods have been reported for the synthesis of common oligonucleotide-polyamide conjugates, based on post-synthetic modification (Ede *et al.* (1994) Bioconj. Chem. 5:373-378; Haralambidis *et al.* (1993) Bioorg. and Med. Chem. Let. 4:1005-1010): assembly of a peptide on controlled pore glass followed by oligonucleotide synthesis (Haralambidis *et al.* (1990) Nuc. Acid. Res. 18:493-499; Haralambidis *et al.* (1987) Tet. Lett. 28:5199-5202; Tong *et al.* (1993) J. Org. Chem. 58:2223-2231; Tung *et al.* (1991) Bioconj. Chem. 2:464-465; Bongratz *et al.* (1994) Nuc. Acid. Res.

22:4681-4688; Zhu and Stein (1994) Bioconj. Chem. 5:312-315) and synthesis of amino modified oligonucleotides followed by solid phase synthesis of peptides.

There are a number of conceivable approaches to the design of oligonucleotide-polyamide conjugates capable of recognizing double helical DNA by triple helix formation. In one approach, the conjugate can be designed such that two minor-groove polyamide oligonucleotide conjugates bind antiparallel to a sequence of duplex DNA, with binding mediated by the dimerization of the individual polyamide moieties in the minor groove of DNA, Figure 20A. In a second approach, the conjugate can be designed such that a single oligonucleotide head-to-tail hairpin polyamide dimer. binds a sequence of duplex DNA in the minor groove, with binding mediated by oligonucleotide directed triple helix formation in the major groove, Figure 20B. In each of these designs specificity is derived from specific contacts in the major groove from the pyrimidine motif triple helix and in the minor groove from the 2:1 polyamide:DNA complex.

BRIEF SUMMARY OF THE INVENTION

The present invention describes a novel method for the preparation of acyclic polyamides containing imidazole and pyrrole carboxamides. The present invention also describes a novel method for the synthesis of cyclic polyamides containing imidazole and pyrrole carboxamides. Further included in the present invention is a novel method for the solid phase synthesis of imidazole and pyrrole polyamide-oligonucleotide and polyamide-protein conjugates capable of recognizing double stranded DNA.

Included in the present invention is the solid phase synthesis of analogs of the di- and tri-N-methylpyrrolecarboxamide antiviral antibiotics Netropsin and Distamycin A. (Figures 2B and 2B).

This invention includes reaction schemes for producing a wide variety of imidazole and pyrrole polyamides and imidazole and pyrrole polyamide-oligonucleotide and protein conjugates. A key element in the synthesis of these compounds is the use of a solid support in conjunction with Boc- (Boc = tert-butoxycarbonyl) and Fmoc- (Fmoc = 9 - fluorenylmethyl carbonyl) chemistry.

More specifically, the invention provides a method for the solid phase synthesis of imidazole and pyrrole polyamides comprising the steps of: preparing a solid support, preferably a polystyrene resin, for attachment of the polyamide to be synthesized; protecting and activating the appropriate amino acid monomers or dimers; sequentially adding the amino acid monomers or dimers to the solid support beginning with the carboxy terminal amino acid; deprotecting the amino acids after formation of the desired polyamide; cleaving the polyamide from the solid support and purifying the synthesized polyamide.

Further included in the present invention are novel amino acid monomers and dimers and novel methods for synthesizing the same.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the basic approach for solid phase peptide synthesis.

Figure 2A depicts the di-N-methylpyrrole-carboxamide antibiotic Netropsin.

Figure 2B depicts the tri-N-methylpyrrole-carboxamide antibiotic Distamycin A.

Figure 3 depicts octapeptide hairpin dimers AcImImPy-γ-PyPyPy-G-Dp (Figure 3A) and AcPyPyPy-γ-ImImPy-G-Dp (Figure 3B), which are designed to bind the sites 5'-WGGWW-3' and 5'-WGGWW-3', respectively. W is either an A,T or T,A base pair.

Figures 4A - 4D illustrate C3-methyl pyrrole inhibition of pyrrole carboxamide-pyrrole carboxamide recognition of a GC base pair. The polyamide oligonucleotide complexes depicted in the Figures are ImPyPy-γ-PyPyPy-Dp•TGTTA and ImPyPy-γ-PyPyPy-Dp•TGTCA. In Figures 4A and 4B the polyamide is unmodified and in Figures 4C and 4D the third pyrrole of the polyamide has been modified by addition of a methyl group to the second carbon of the pyrrole ring.

Figure 5 illustrates the potential for the formation of a bifurcated hydrogen bond between a 3-substituted hydroxypyrrole carboxamide and the carbonyl of thymine (dR represents deoxyribose).

Figure 6A illustrates the additional hydrogen bond which can form between the hydroxyl group of a 3-substituted hydroxypyrrole carboxamide and the carbonyl of thymine.

Figure 6B illustrates that adenine cannot form this additional hydrogen bond.

Figure 7 depicts illustrative polyamides prepared by the method of this invention.

Figure 8 illustrates a typical 72 minute solid phase synthesis cycle for minor groove polyamides according to one embodiment of this invention.

Figure 9 illustrates three representative analytical high pressure liquid chromatography (HPLC) traces for stepwise monitoring of a solid phase polyamide synthesis cycle from the synthesis of AcImImPy-γ-PyPyPy-G-Dp (0.1% wt/v TFA, gradient elution 1.25% CH_3CN/min monitored at 254 nm). Figure 9A depicts the HPLC spectrum of Boc-Py-γ-PyPyPy-G-Dp (Boc = tert-butoxycarbonyl) which elutes at 31.6 minutes. Figure 9B depicts the spectrum of H_2N-Py-γ-PyPyPy-G-Dp which elutes at 24.3 minutes and Figure 9C depicts the spectrum of Boc-ImPy-γ-PyPyPy-G-Dp which elutes at 31.8 minutes.

Figure 10 illustrates various spectra of the HPLC purified polyamide, AcImImPy-γ-PyPyPy-G-Dp (**4a**) (Scheme 2). Figure 10A depicts the HPLC spectrum (0.1% wt/v TFA, 1.25% CH_3CN/min). monitored at 254 nm. Figure 10B depicts the MALDI-TOF mass spectrum. internal standard at 1802.1 (M^+H calculated for $C_{47}H_{60}N_{18}O_9$, 1022.1, found 1022.4). Figure 10C depicts the ^1H NMR spectra recorded at 300 MHz in d_6-DMSO.

Figure 11 depicts ribbon models of "slipped" (**11A**) and "overlapped" (**11B**) 2:1 polyamide:DNA complexes.

Figures 12A - 12H depict graphically the data obtained from the quantitative DNase I footprint titration experiments. The (θ_{norm}, $[L]_{tot}$) data points were obtained as described in Example 10.

Figure 13 depicts the structures of the C-termini of various polyamides illustrating that polyamides with C-termini (a) Py-Dp and (b) Py-β-Dp (β = β-alanine) sequences present similar surfaces to the minor groove, while polyamides with C-termini (c) Py-G-Dp sequences present a different surface. In the case of (c) the glycine carbonyl group is directed toward the minor groove.

Figure 14 illustrates the predicted 2:1 complexes of ImPyPy-X-PyPyPy-G-Dp, where X = G. β or Py, with the targeted sites (a) 5'-AAAAAGACAAAAA-3' (SEQ ID NO:2), (b) 5'-ATATAGACATATA-3' (SEQ ID NO:3) (13 bp, "slipped") and (c) 5'-TGTTAAACA-3' (SEQ ID NO:4) (9 bp, "overlapped"). The shaded and light circles represent imidazole and pyrrole rings, respectively, and the diamond represents the internal amino acid X. The specifically targeted guanines are highlighted.

Figure 15 depicts the storage phosphor autoradiograms of the 8% denaturing polyacrylamide gels used to separate the fragments generated by DNase I digestion in the quantitative footprint titration experiments (Example 10). The five binding sites analyzed in the footprint titration experiments are indicated on the right sides of the autoradiogram.

Figure 16 depicts graphically the measurement of the time of the coupling of Boc-Py-OBt to $PyNH_2$ by picric acid titration. Samples were taken at one minute intervals.

Figure 17 illustrates two ways in which double helical DNA can be recognized by an oligonucleotide-minor groove binding polyamide conjugate. In Figure 17A oligonucleotide directed triple helix formation in the major groove is mediated by polyamide dimerization in the minor groove. In Figure 17B directed binding of a head-to-tail polyamide dimer in the minor groove is mediated by oligonucleotide directed triple helix formation in the major groove.

Figure 18 depicts illustrative polyamide-oligonucleotide conjugates synthesized by the method of this invention. Included in the figure are the calculated and observed masses (MALDI-TOF).

Figure 19 depicts a ribbon graphic illustrating how the oligonucleotide-polyamide conjugate Dp-G-PyPyPy-G-PyPyIm-linker-TTTTTT^{m}C^{m}CTTT might bind to double helical DNA.

Figure 20 illustrates a ribbon model of the GCN-4 protein's coiled and DNA binding region binding to DNA.

Figure 21 depicts a ribbon model of the GCN-4-polyamide conjugate illustrating the binding region of the substituted polyamide to DNA.

Figure 22 depicts a schematic representation of a peptide synthesizer.

Figure 23 depicts a flow chart of the computer program used to produce polyamides in the peptide synthesizer illustrated in Figure 22.

Figure 24 depicts a ball and stick model of the projected binding mode of polyamide H_2N-β-PyPyPy-γ-ImImPy-β-β-β-β-PyPyPy-γ-ImImPy-β-Dp with the target DNA sequence 5'-TGGTTAGTACCT-3' (SEQ ID NO:5).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for the solid phase synthesis of straight chain and cyclic polyamides containing imidazole and pyrrole carboxamides. The present invention also provides a method for the solid phase synthesis of imidazole and pyrrole polyamide-oligonucleotide conjugates and imidazole and pyrrole polyamide-protein conjugates.

Included in the present invention is the solid phase synthesis of analogs of the di- and tri-N-methylpyrrolecarboxamide antibiotics Netropsin and Distamycin A (Figures 2A and 2B).

More specifically the present invention includes a method for the preparation and identification of pyrrole and imidazole carboxamide polyamides and polyamide-oligonucleotide and protein conjugates which recognize double stranded DNA by interaction with the minor groove of the DNA.

Illustrative imidazole and pyrrole polyamides produced by the method of this invention are shown in Figure 7 and Tables 1 and 4. Illustrative

imidazole and pyrrole carboxamide polyamide-oligonucleotide conjugates produced by the method of this invention are shown in Table 5 and Figure 18.

The present invention extends to all novel imidazole and pyrrole carboxamide polyamides, imidazole and pyrrole carboxamide polyamide-oligonucleotide conjugates and imidazole and pyrrole carboxamide polyamide-protein conjugates that can be prepared according to the methods of the present invention.

Further included in this invention is an improved method for the preparation of the Boc-pyrrole-OBt and Boc-imidazole-OBt activated amino acid monomers and a novel method for the preparation of the Fmoc-pyrrole-OBt and Fmoc-imidazole-OBt activated amino acid monomers. Also included in this invention are novel monomers in which the pyrrole is substituted at the *N*-methyl position and at the 3 position of the pyrrole ring. Also included is a novel method for the preparation of imidazole containing dimers.

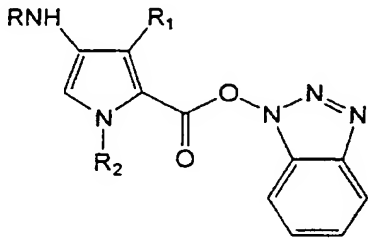
Certain terms used to describe the invention herein are defined as follows:

The term "polyamide" is used to describe the polypeptides synthesized by the method of this invention. A polyamide is a polymer of amino acids chemically bound by amide linkages (CONH). An "amino acid" is defined as an organic molecule containing both an amino group (NH_2) and a carboxylic acid (COOH). The polyamides of this invention are comprised of imidazole carboxamides, pyrrole carboxamides, aliphatic amino acids, aromatic amino acids and any chemical modifications thereof.

The term "amino acid monomer" refers to a pyrrole or imidazole amino acid or an aliphatic or aromatic amino acid in which the amine has been protected with the Boc- protecting group, Fmoc- protecting group or allyl-

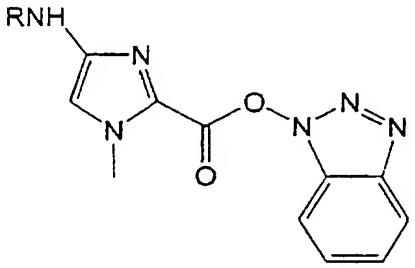
protecting group and the carboxylic acid has been activated as the -OBt ester or the symmetric anhydride.

The activated "pyrrole amino acid monomers" of this invention are generally depicted as follows:



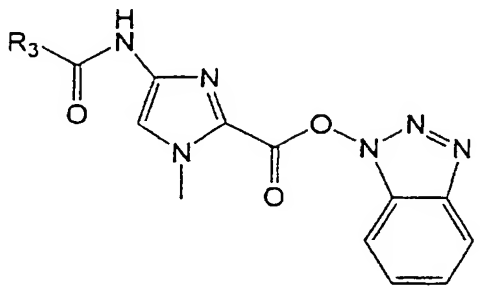
wherein R is a protecting group independently selected from the groups consisting of tert-butoxycarbonyl (Boc-), allyl (-$CH_2CH=CH_2$) or 9-fluoroenylmethyl carbonyl (Fmoc-); R_1 is independently selected from the group consisting of H, CH_3, OH, NH_2, Cl or CF_3; and R_2 is independently selected from the group consisting of H, C1-C10 alkyl, such as methyl, ethyl or isopropyl, C1-C10 alkenyl, C1-C10 alkynyl, such as -$C{\equiv}CCH_3$, or a carboxylic acid, such as -CH_2COOH.

The activated "imidazole amino acid monomers" of this invention are generally depicted as follows:



wherein R is a protecting group independently selected from the groups consisting of tert-butoxycarbonyl (Boc-), allyl (-$CH_2CH=CH_2$) or 9-fluoroenylmethylcarbonyl (Fmoc-).

The -OBt activated "dimers" of this invention are generally depicted as follows:



wherein R_3 is independently selected from the group consisting of an aliphatic or aromatic amino acid. an imidazole carboxamide or a pyrrole carboxamide or any chemical modification thereof.

"Oligonucleotide-polyamide conjugate" is a term used to describe a molecule which is comprised of an oligonucleotide chain and a polyamide chain joined by a covalent linkage.

"Protein-polyamide conjugate" is a term used to describe a molecule which is comprised of a protein chain and a polyamide chain joined by a covalent linkage.

"Nucleoside" means either a deoxyribonucleoside or a ribonucleoside or any chemical modifications thereof. Modifications of the nucleosides include, but are not limited to, 2'-position ribose modifications, 5-position pyrimidine modifications, 8-position purine modifications, modifications at cytosine exocyclic amines and the like.

A "failure sequence" refers to a polyamide chain which has not reacted with the pyrrole/imidazole monomer or dimer during a particular reaction cycle.

The solid phase polyamide synthesis protocols of this invention were modified from the *in situ* neutralization. Boc- chemistry recently reported

by Kent and coworkers (Schnolzer *et al.* (1992) Int. J. Peptide Protein Res. 40:180-193; Milton *et al.* (1992) Science 252:1445-1448). In its most basic form the method of preparing imidazole and pyrrole carboxamide polyamides according to the present invention may be defined by the following series of steps: (1) The solid support, preferably a polystyrene resin, is prepared. The polystyrene resin is prepared by reaction with a linker molecule to enable facile attachment and removal of the polyamide. In one embodiment a spacer molecule is attached to the polyamide prior to attachment of the linker molecule. (2) The appropriate amino acid (aa) monomer or dimer is then protected at the amino (NH_2) group and activated at the carboxylic acid (COOH) group. The amino (NH_2) group is protected with a Boc- group an Fmoc- group and the carboxylic acid is activated by the formation of the -OBt ester, to give, in the case of the pyrrole and imidazole amino acids Boc-pyrrole-OBt (**9**), Boc-imidazole-OBt (**13**), Fmoc-pyrrole-OBt (**21a**) and Fmoc-imidazole-OBt (**21b**). (3) The protected and activated amino acids are then sequentially added to the solid support beginning with the carboxy terminal amino acid. High concentrations of activated monomer results in fast coupling reactions and *in situ* neutralization chemistry assures that the unstable deprotonated amine is generated simultaneous with the initiation of a coupling reaction. Coupling times are rapid, generally 72 minutes per residue, and simple, requiring no special precautions beyond those required for ordinary solid phase peptide synthesis. (4) When the desired polyamide has been prepared the amino acids are deprotected and the peptide is cleaved from the resin and purified. The reactions are periodically monitored using picric acid titration and high pressure liquid chromatography (HPLC). Each of these steps are described in detail below. The synthesis of ImPyPyPyPyPy-G-ED (G =

glycine, ED = ethylenediamine) **1a**, ImPyPyPyPyPyPy-G-Dp (Dp = dimethylaminopropylamine) **1b**, ImPyPyPyPyPyPy-G-Ta (Ta = 3,3'-diamino-N-methylpropylamine) **1c**, ImPyPyPyPyPyPy-G-Ta-EDTA (EDTA = ethylenediaminetetraacetic acid) **1d**, ImPyPy-G-PyPyPy-G-ED **2a**, ImPyPy-G-PyPyPy-Dp **2b**, AcImPyPy-G-PyPyPy-G-Dp (Ac = acyl) **2c**, AcImPyPy-G-PyPyPy-G-Ta-EDTA **2d**, AcImPyPy-γ-PyPyPy-G-Dp **3a**, AcImPyPy-γ-PyPyPy-G-Ta **3b**, AcImPyPy-γ-PyPyPy-G-EDTA **3c**, AcImImPy-γ-PyPyPy-G-Dp **4a**, AcImImPy-γ-PyPyPy-G-Ta **4b**, AcImImPy-γ-PyPyPy-G-EDTA **4c**, AcPyPyPy-γ-ImImPy-G-Dp **5a**, AcPyPyPy-γ-ImImPy-G-Ta **5b**, and AcPyPyPy-γ-ImImPy-G-Ta-EDTA **5c** (Figure 7) is described herein. A complete list of illustrative polyamides synthesized by the methods of this invention is set forth in Table 1. All compounds listed in this table have been characterized by ^{1}H NMR, HPLC, MALDI-TOF mass spectroscopy and in some cases ^{13}C NMR.

The pyrrole and imidazole polyamides of this invention are contemplated for use as antiviral, antibacterial and antitumor compounds which recognize double stranded DNA by interaction with the minor groove of the DNA. Specifically, it is anticipated that the pyrrole and imidazole polyamides may be used to sequence DNA ligands which are able to specifically inhibit DNA binding proteins, such as transcription factors which are responsible for gene regulation, thus, providing a basis for rapid rational design of therapeutic compounds. The ethylenediaminetetraacetic acid (EDTA) derivatives of the polyamides synthesized by the method of this invention are also contemplated for use in the field of molecular biology. These molecules can be used to bind and cleave double stranded DNA at a specific site using iron (Fe) and EDTA.

It is further contemplated that the novel *N*-substituted pyrrole monomers of this invention will provide polyamides with novel DNA binding properties, with enhanced pharmacological properties, or provide functionalized polyamides for synthesis of modified derivatives.

In its most basic form the method of preparing imidazole and pyrrole polyamide-oligonucleotide conjugates according to the present invention may be defined by the following series of steps: (1) The oligonucleotide is assembled on a solid support using standard methodology. (2) The appropriate amino acid monomer is then protected and activated. The amino group is protected with the Boc- or Fmoc- group and the aromatic acid is activated by the formation of the -OBt ester, to give, in the case of the pyrrole and imidazole amino acids, Boc-pyrrole-OBt (**9**) and Boc-imidazole-OBt (**13**) Fmoc-pyrrole-OBt (**21a**) and Fmoc-imidazole-OBt (**21b**). (3) The protected and activated amino acids are then sequentially added to the assembled oligonucleotide beginning with the carboxyterminal amino acid. As stated above, high concentrations of activated monomer results in fast coupling reactions and *in situ* neutralization chemistry assures that the unstable deprotonated amine is generated simultaneous with the initiation of a coupling reaction. Coupling times are rapid, generally 72 minutes per residue, and simple, requiring no special precautions beyond those required for ordinary solid phase peptide synthesis. (4) When the desired polyamide has been prepared the amino acids are deprotected and the polyamide-conjugate is cleaved from the resin.

The pyrrole and imidazole polyamide-oligonucleotide conjugates of this invention are contemplated for use as potential antiviral compounds which recognize double stranded DNA by triple helix formation. Many DNA-

binding proteins bind in the major groove of DNA. It is anticipated that polyamide-oligonucleotide conjugates may be more effective inhibitors of sequence specific DNA binding proteins, since they will occlude both the major and minor grooves.

In its most basic form the method of preparing imidazole and pyrrole polyamide-protein conjugates according to the present invention may be defined by the following series of steps: (1) The protein is assembled using standard methodology; (2) The appropriate amino acid monomer is then protected and activated as discussed above. (3) The protected and activated amino acid monomers are then sequentially added to the assembled protein beginning with the carboxyterminal amino acid. (4) When the desired polyamide has been prepared the amino acids are deprotected and the polyamide-conjugate is cleaved from the resin.

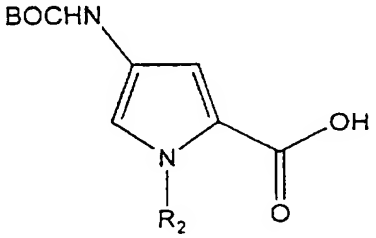
The pyrrole and imidazole polyamide-protein conjugates of this invention are contemplated for use as potential antiviral, antibacterial and antitumor compounds which recognize double stranded DNA by interaction with the minor groove of DNA. Many DNA-binding proteins bind in the major groove of DNA. It is anticipated that the appended peptide moiety will provide a means for introducing the polyamide into the cell.

Synthesis of the Imidazole and Pyrrole Amino Acid Monomers. The Boc-pyrrole-OBt (Boc-Py-OBt) (**9**) and Boc-imidazole-OBt (Boc-Im-OBt) (**13**) monomers are synthesized using a modified procedure of Grehn and coworkers (Grehn and Ragnarsson (1991) J. Org. Chem. 46:3492-3497; Grehn *et al.* (1990) Acta. Chem. Scand. 44:67-74) (Example 1 and Scheme 9). The modification involves the use of the commercially available Boc-anhydride (di-*t*-butyl-dicarbonate) as the Bocing agent, rather than the highly reactive Bocing

agent, tert-butyloxycarbonyl fluoride (Boc-fluoride) employed by Grehn. Boc-fluoride is dangerous to prepare in large quantities, requiring the use of chlorofluorophosgene which is very toxic. Additionally, Boc-fluoride is not stable for storage (Wackerle and Ugi (1975) Synthesis 598-599; Franzen and Ragnarsson (1979) Acta. Chem. Scand. 33:690-692; Dang and Olofson (1990) J. Org. Chem. 55:1847-1851). The reaction of Boc-anhydride with the pyrrole amino group has been reported by Bailey *et al.* (1989) J. Pharm. Sci. 78: 910-917. Overall yields starting from the nitro/methyl esters are reproducibly greater than 60% for both the pyrrole and imidazole -OBt esters, with simple purification requiring no column chromatography. Additionally, the Boc-imidazole-OBt ester prepared by this procedure is stable at room temperature.

The Fmoc- protected monomers are synthesized from the Boc-protected monomers as illustrated in Scheme 12 (Example 1). Fmoc- is an alternate protecting group commonly used for peptide synthesis. Fmoc- is removed with dilute base, whereas the Boc- group is removed under acidic conditions. The use of Fmoc- as a protecting group provides additional versatility to the method of this invention.

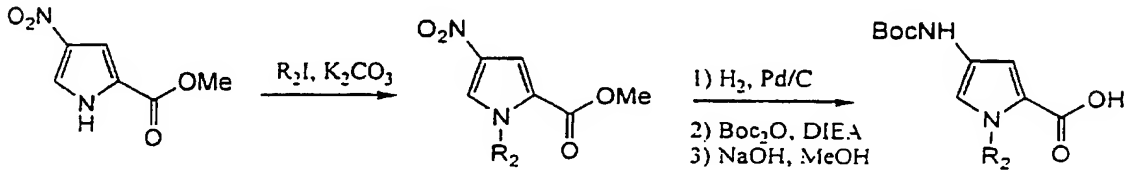
Boc-pyrrole monomers substituted at the *N*-methyl position having the following general formula:



where R_2 is independently selected from the group consisting of H, C1-C10 alkyl, such as methyl, ethyl or isopentyl, a 1° or 2° amine, such as N, N,

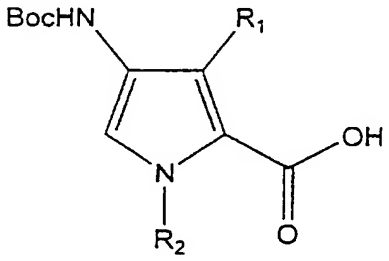
dimethylpropylamine, ethylamine, a carboxylic acid, such as -CH_2-COOH, an alkenyl, or an alkynyl, such as -≡-CH_3 are prepared as illustrated in Scheme 1. As stated above, it is believed that such compounds will provide polyamides with enhanced pharmacological properties. A general method for the preparation of these compounds is as follows:

Scheme 1



(*See* Example 1, Scheme 13).

Pyrrole monomers substituted at the 3 position of the pyrrole ring having the following general formula:



wherein R_1 is independently selected from the group consisting of a Cl-C10 alkyl group, such as -CH_3, or an -OH, -NH_2, or -OR_4, wherein R_4 is a Cl-C10 alkyl group, such as methyl or allyl and R_2 is independently selected from the group consisting of H, Cl-C10 alkyl, such as methyl, ethyl or isopentyl, a 1° or 2° amine, such as N, N. dimethylpropylamine, ethylamine, a carboxylic acid,

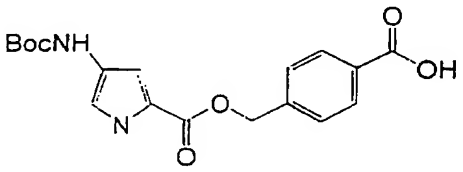
such as $-CH_2-COOH$, an alkenyl, or an alkynyl, such as $-\equiv-CH_3$, were synthesized as discussed in Example 1 (Schemes 14 and 15). Such compounds are of interest because they allow substitution of a proton which is known by structural studies to be tightly associated in the floor of the minor groove (*see* Figure 4). As discussed below, monomers substituted at the 3 position of the pyrrole ring will likely provide polyamides with novel DNA binding properties.

While studying polyamide DNA complexes, it was discovered that the recognition of a GC base pair by the combination of a pyrrole carboxamide opposite a pyrrole carboxamide is a potential mismatch. (Figure 4C) (Mirksich *et al.* (1994) J. Am. Chem. Soc. 116:6873-7988). A polyamide prepared using a pyrrole monomer substituted at the 3 position with an alkyl group, therefore, will likely introduce a steric clash when pyrrole carboxamide/pyrrole carboxamide binds opposite GC. (Figure 4D). A.T recognition by the methyl derivative will not be greatly inhibited. (Figure 4B). A general method for the preparation of a pyrrole with an alkyl substituent in the 3 position is set forth in Example 1 (Scheme 14).

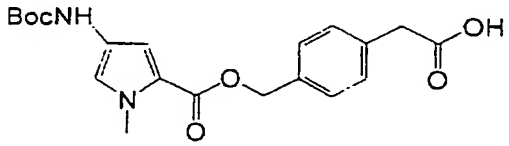
Substituted monomers in which R_1 is -OH or $-NH_2$ may be used to introduce a specific hydrogen bond interaction between the -OH or $-NH_2$ group of the pyrrole and the carbonyl of thymine, which is capable of forming bifurcated hydrogen bonds, and should be able to hydrogen bond to both a pyrrole carboxamide and the substituted pyrrole. Adenine, on the other hand, will not be able to form this additional hydrogen bond, since it has only a single lone pair electron which is already hydrogen bonded to a pyrrole carboxamide. (*See* Figures 5 and 6). A general method for the preparation of a pyrrole with a

hydroxy or alkoxy substituent in the 3 position of the ring is set forth in Example 1 (Scheme 15).

Synthesis of Resin Linkage Agents. For solid phase synthesis, the growing polymer chain must be attached to the insoluble matrix by a linkage agent which is stable for the course of the synthesis, but cleaved in high yields under appropriate conditions to release the synthesized polymer. A number of resin linkages were investigated. Of these, Merrifield's PAM (tert-butyloxycarbonylaminoacyl-pyrrole-4-(oxymethyl)phenyl-acetamidomethyl resin (Boc-Py-PAM-resin), synthesized as described below, is the preferred solid support because it is stable to trifluoroacetic acid (TFA) and because it can be cleaved from the resin under a variety conditions including, liquid hydrogen fluoride, amminolysis, hydrolysis, hydrazinolysis, catalytic hydrogenation, or lithium borohydride to give a peptide acid, amide, hydrazide, or primary alcohol, as discussed above. Two resin linkage agents, compounds **39** and **40** were employed. These compounds were prepared in three steps according to the published procedures of Merrifield, using the Boc- protected pyrrole amino acid, Boc-Py-COOH (**8**) (Mitchell *et al.* (1978) J. Org. Chem. 43:2845-2852) (Example 3, Scheme 16).



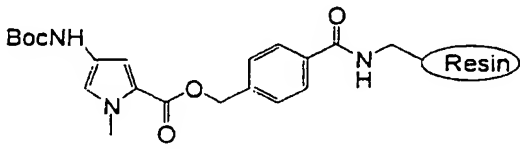
(39)



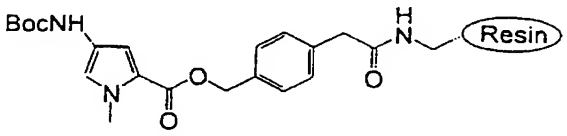
(40)

To attach the resin linkage agents to the resin, compounds **39** and **40** were activated with dicyclohexylcarbodiimide (DCC) followed by reaction with aminomethylated polystyrene for 24 hours to give Boc-pyrrole-BAM-resin **41**

and Boc-pyrrole-PAM-resin **42**, respectively (Example 3). The reactions were preferably stopped at 0.2 to 0.3 mmol/gram substitution as determined by the quantitative ninhydrin test and by picric acid titration (Sarin *et al.* (1981) Anal. Biochem. 117:147-157; Gisin (1972) Anal. Chim. Acta. 58:248).

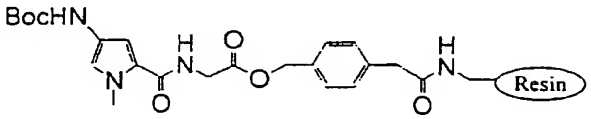


Boc-Py-BAM-resin
(41)

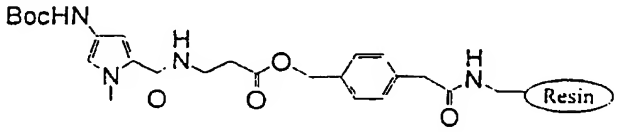


Boc-Py-PAM-resin
(42)

Activated resin linkage agents **39** and **40** were also reacted with the commercially available Boc-glycine-PAM-resin (Boc-G-PAM-resin) to give Boc-pyrrole-PAM-G-PAM-resin (Boc-Py-PAM-G-PAM-resin) and the corresponding BAM resin. Finally, the commercially available Boc-G-PAM-resin and Boc-β-alanine-PAM-resin (Boc-β-PAM-resin) were reacted with Boc-Pyrrole-OBt (**9**) to yield the Boc-pyrrole-G-PAM-resin (**43**) (*see* Scheme 2, step a) and the Boc-pyrrole-β-PAM-resin (**44**), respectively. Unreacted amino groups were capped by acetylation.



Boc-Py-G-PAM-resin
(43)



Boc-Py-β-PAM-resin
(44)

The corresponding Boc-imidazole-G-PAM and Boc-imidazole-β-PAM-resins were synthesized using the same procedure.

Solid Phase Polyamide Synthesis Protocols. The solid phase peptide synthesis (SPPS) methods of this invention were modified from the *in situ* neutralization chemistry described by Kent and coworkers. (Schnolzer *et al.* (1992) Int. J. Peptide Protein Res. 40:180-193; Milton *et al.* (1992) Science 252:1445-1448). One embodiment for the synthesis of polyamides according to the method of this invention is listed in Table 2 and shown schematically in Figure 8. (*See* Example 4). The Boc- protected monomers are used in Example 4 for purposes of illustration. The methods illustrated are readily extendable to the Fmoc- protected monomers.

The method consists of washing either the Boc-pyrrole-PAM/BAM-resins (**41** and **42**), or the Boc-pyrrole-G-PAM (**43**)/Boc-pyrrole-β-PAM (**44**) resins or the corresponding Boc-imidazole resins with dichloromethane followed by the removal of the Boc- group with 65% trifluoroacetic acid (TFA)/ 35% dichloromethane/ 0.5M thiophenol for 20 minutes. The deprotected resin is then washed with dichloromethane followed by dimethylformamide (DMF). A sample of the resin can be analyzed at this time using the picric acid test, as described below. After deprotection of the resin, an -OBt activated amino acid monomer and diisopropylethylamine are added to the resin and the reaction is allowed to proceed for 45 minutes. After 45 minutes a sample is taken for analysis and the resin is washed with DMF. The overall stepwise assembly of a single residue takes approximately 72 minutes under the standard conditions.

The coupling of the -OBt ester of Boc-pyrrole to the imidazole amine on the solid support was found to be slow using the above procedure. In

a preferred embodiment of the invention, the pyrrole monomer was activated as the symmetric anhydride, rather than the -OBt ester, using a modified procedure of Ding *et al.* (1963) Acta Chem. Scand. 23:751. Ding describes the solution phase coupling of pyrrole to pyrazole by formation of the symmetric anhydride of the pyrrole monomer (DCC and DMAP in dichloromethane) prior to coupling. This procedure was modified for use on a solid support and provides a rapid and effective method for the coupling of pyrrole to imidazole. This modified procedure, which is described in Example 5, provides a high yielding rapid reaction. Coupling yields were found to be greater than 98% for the synthesis of the polyamide AcPyImPy-G-Dp.

In developing a method for the solid phase synthesis of polyamides, it was anticipated that intermolecular chain aggregation could be a severe problem, with the adjacent extended aromatic polyamide providing excellent surface areas for stacking. To minimize the possibility of aggregation, a low substitution resin, 0.2 mmol or 0.3 mmol per gram, is used in combination with *in situ* neutralization. The high coupling yields (>99 % in most cases), indicate that intermolecular interactions are not a problem under these conditions. *In situ* neutralization has also been found to increase the lifetime of the aromatic amines.

In situ neutralization involves the elimination of a separate neutralization step by adding diisopropylethylamine (DIEA) simultaneously with the activated monomer. In standard solid phase peptide synthesis it has been demonstrated by direct physical measurement that intermolecular aggregation of the growing peptide chain is disrupted by TFA, which is an excellent solvent for most peptides, the aggregates reform, however, during the subsequent neutralization of the amine trifluoroacetate (Larsen *et al.* (1990)

Peptides 183-185; Woerkom and Nipsen (1991) Int. J. Pep. Prot. Res. 38: 103-113; Milton *et al.* (1990) J. Am. Chem. Soc. 112:6039-6046). By adding the monomer simultaneously with neutralizing agent, coupling is able to occur before an aggregate can form. *In situ* neutralization was adopted by Kent and coworkers to eliminate low coupling yields resulting from intermolecular aggregation in Boc-chemistry SPPS. (Larson *et al.* (1990) Peptides 183-185; Hudson (1988) J. Org. Chem. 53:617-624; Woerkom and Nipson (1991) Int. J. Pep. Prot. Res. 38:103-113).

In the standard *in situ* neutralization procedure DMF is used as the solvent, because it maximizes the solvation of the growing peptide chain. Because the mixing of DMF and TFA is very exothermic the standard procedure of Kent is modified by addition of the steps of washing the resin with dichloromethane both before and after treatment with TFA. Thiophenol is also added in the TFA deprotection step as a scavenger for the t-butyl cation. This is necessitated by the potential for side reactions between the t-butyl cation and the unprotected imidazole nitrogen, which has been reported to be nucleophilic in solution phase reactions. (Grehn and Ragnarsson (1981) J. Org. Chem. 46:3492-3497; Grehn *et al.* (1990) Acta. Chem. Scand. 44:67-74). Thiophenol, methyl ethyl sulfide, and ethanedithiol have all been reported to compete effectively as scavengers of t-butyl cation. (Lundt *et al.* (1978) Int. J. Pep. Prot. Res. 12:258-268).

Monitoring the Progress of the Synthesis. In standard SPPS, the quantitative ninhydrin test is the preferred method of monitoring the coupling reactions and calculating yields. (Sarin *et al.* (1981) Anal. Biochem. 117:147-157: Gisin (1972) Anal. Chim. Acta. 58:248). The aromatic amines of pyrrole and imidazole. however. do not react in the quantitative ninhydrin test. In

place of the ninhydrin test, picric acid titration and stepwise cleavage and monitoring by HPLC are used to estimate coupling yields and monitor the course of the reactions. In the few cases where it is possible to use the quantitative ninhydrin test, such as the coupling of Boc-Py-OBt to N_2H-γ or H_2N-G, all yields have been observed to be better than 99.8%.

Picric acid titration measures the amount of unreacted amine remaining. The method involves formation of the picrate salt of the amine, which is then quantitated from the reported extinction coefficients. The experimental procedure is set forth in Example 6. The picric acid test is inaccurate for low concentrations of amine, thus it is only possible to determine if a reaction is >90% complete using this measurement. Picric acid titration is useful for immediate monitoring of coupling reactions.

High pressure liquid chromatography (HPLC) is used for the stepwise monitoring of the polyamide synthesis. After each coupling reaction a small portion of resin is removed from the reaction mixture and the polyamide is cleaved from the resin and analyzed by analytical HPLC, as described in Example 7. The use of stepwise HPLC analysis is an effective way to obtain detailed information on the progress of a synthesis, allowing the exact step that results in a side reaction or deletion product to be readily identified and eliminated.

Cleavage of the Polyamide from the Boc-pyrrole-resin. Example 8 describes a general method for cleaving the polyamide from the Boc-Py-PAM/BAM-resins using $Pd(OAc)_2$. The successful cleavage of minor groove polyamides is achieved from PAM and BAM pyrrole resins with $Pd(OAc)_2$ in DMF under a pressurized atmosphere of hydrogen (100 psi, 8 hours). Scheme 17 (Example 8) illustrates this procedure with the acetylated tripyrrole

AcPyPyPy-PAM-resin. Upon being cleaved from the resin the terminal pyrrole acid can be activated with DCC/hydroxybenzotriazole (HOBt) and reacted with a primary amine to yield the corresponding amide.

Cleavage from PAM and BAM resins by amminolysis was unsuccessful at 37°C and 60°C in 1:1 amine:DMF or neat amine for 24 hours.

Cleavage of the Polyamide from the Boc-G-PAM-Resin. Example 9 describes a general method for the cleavage of the polyamide from the Boc-G-PAM-resin using a 1:1 mixture of dimethylaminopropylamine:DMF. Scheme 18 (Example 9) illustrates this method with the acetylated tripyrrole AcPyPyPy-PAM-G-PAM-resin. After reaction with a 1:1 mixture of dimethylaminopropylamine:DMF at 37°C for 12 hours, two products AcPyPyPy-PAM-G-Dp (98% of product) and the failure sequence AcPyPy-PAM-G-Dp (2%) were identified by ^{1}H NMR. Recovery of the product was very high --almost 50% of the theoretical yield-- indicating that the pyrrole-G-PAM-resin is cleaved with much higher recovery than the pyrrole-PAM-resin.

Based on the excellent recovery of acetylated tripyrrole under chemically mild conditions, the use of a glycine spacer is the preferred synthetic method. This method offers two advantages, high cleavage yields from the resin and commercial availability of highly pure Boc-G-PAM-resin with 0.2 mmol/gram substitution.

Purification and Characterization of Peptides. Reversed phase HPLC purification provides a convenient and efficient method for the purification of the solid phase peptide products (Fransson *et al.* (1983) J. Chrom. 268:347-351). Amminolysis reactions are filtered to remove the resin, diluted with water and immediately purified by reversed phase HPLC with a

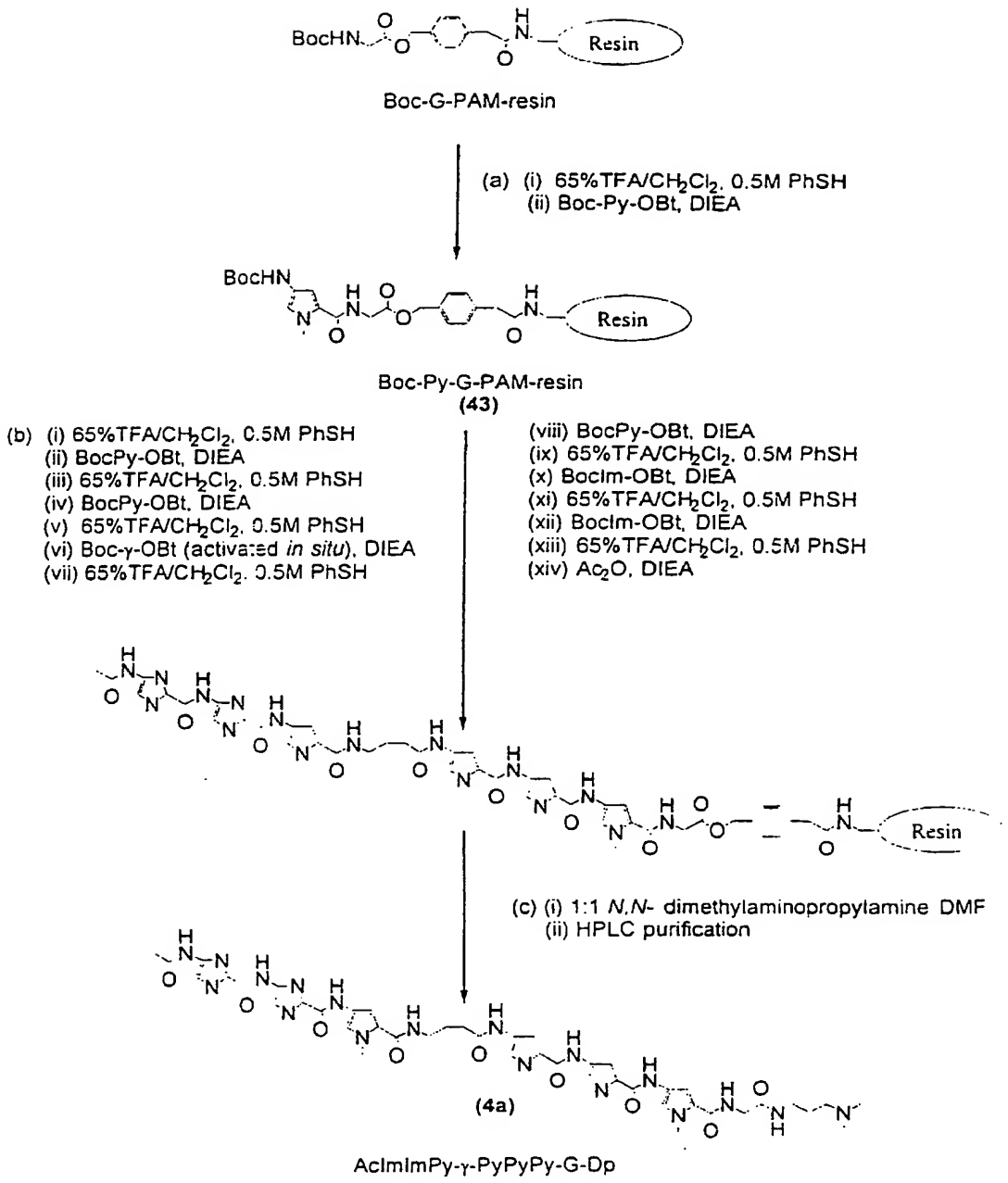
gradient of 0.15% CH_3CN/min. in 0.1% wt./v. TFA. A single preparatory run is sufficient to obtain purity greater than 98% as determined by a combination of HPLC, ^1H NMR and mass spectroscopy. ^1H NMR is carried out in the non-exchangeable solvent d_6-DMSO, making all amide hydrogens and the trifluoroacetamide protons clearly visible. 2-D COSY experiments are used to assist in the assignment of protons. MALDI-TOF mass spectroscopy provides an accurate and rapid method of confirming that full length product has been isolated.

Scheme 2 illustrates a representative solid phase synthetic scheme for the polyamide AcImImPy-γ-PyPyPy-G-Dp (Dp = dimethylaminopropylamine) (**4a**) starting from the commercially available Boc-G-PAM-resin. The polyamide was synthesized with 7 standard synthesis cycles and the final product was acetylated. The synthesis was monitored by analytical HPLC as illustrated in Figures 9A - 9C. Figure 9A, which depicts the spectrum of Boc-Py-γ-PyPyPy-G-Dp, shows that the synthesis has proceeded after five reaction cycles with a major peak eluting at 31.6 minutes observed. The Boc- group is removed under standard conditions to give upon cleavage of a small sample H_2N-Py-γ-PyPyPy-G-PAM-resin which elutes at 24.3 minutes (Figure 9B). The pyrrole amine is reacted with 4 equivalents of Boc-Im-OBt under standard conditions, giving a quantitative conversion to Boc-ImPy-γ-PyPyPy-G-PAM-resin which is observed upon cleavage with dimethylaminopropylamine to elute at 31.8 minutes (Figure 9C). All coupling reactions proceeded in greater than 90% yield as determined by picric acid titration. The coupling of pyrrole to γ-aminobutyric acid (γ) and glycine proceeded in 99.9% yield as determined by the quantitative ninhydrin test. All yields are established as >99% by HPLC analysis of each individual coupling

step. Monitoring each individual step before and after deprotection assures that high yields are being obtained. Upon completion of the synthesis, the resin is cleaved by amminolysis with a 1:1 mixture of DMF and *N,N*-dimethylaminopropylamine at 37°C for 12 hours. After 12 hours the reaction mixture is filtered to remove the resin, diluted with 4 volumes water and immediately purified by reversed phase HPLC with a gradient of 0.15% CH_3CN/min. in 0.1% wt/v TFA. A single preparatory scale separation is sufficient to obtain purity greater than 98% as determined by a combination of HPLC, ^1H NMR and mass spectroscopy.

The HPLC, MS and ^1H NMR spectra of the purified product are shown in Figures 10A-10C respectively. As can be seen in the ^1H NMR spectrum (Figure 10C) only the expected protons are observed, from high field to low field, 2 imidazole carboxamide protons, four pyrrole carboxamide protons, a trifluoroacetate proton (the tertiary amine is obtained as the trifluoroacetate salt after HPLC purification in 0.1% TFA. and the trifluoroacetate proton is identified by 2-D COSY experiments), the three amides corresponding to the aliphatic amines, two imidazole ring protons (singlets) and 8 pyrrole ring protons are observed as either doublets or multiplets. MALDI-TOF mass spectroscopy (Figure 10B) provides an accurate and rapid method of confirming that full length product has been isolated and that side reactions such as alkylation or acylation of the unprotected imidazole nitrogen have not occurred. A combination of analyses ensures that pure full length peptide has been obtained in high purity.

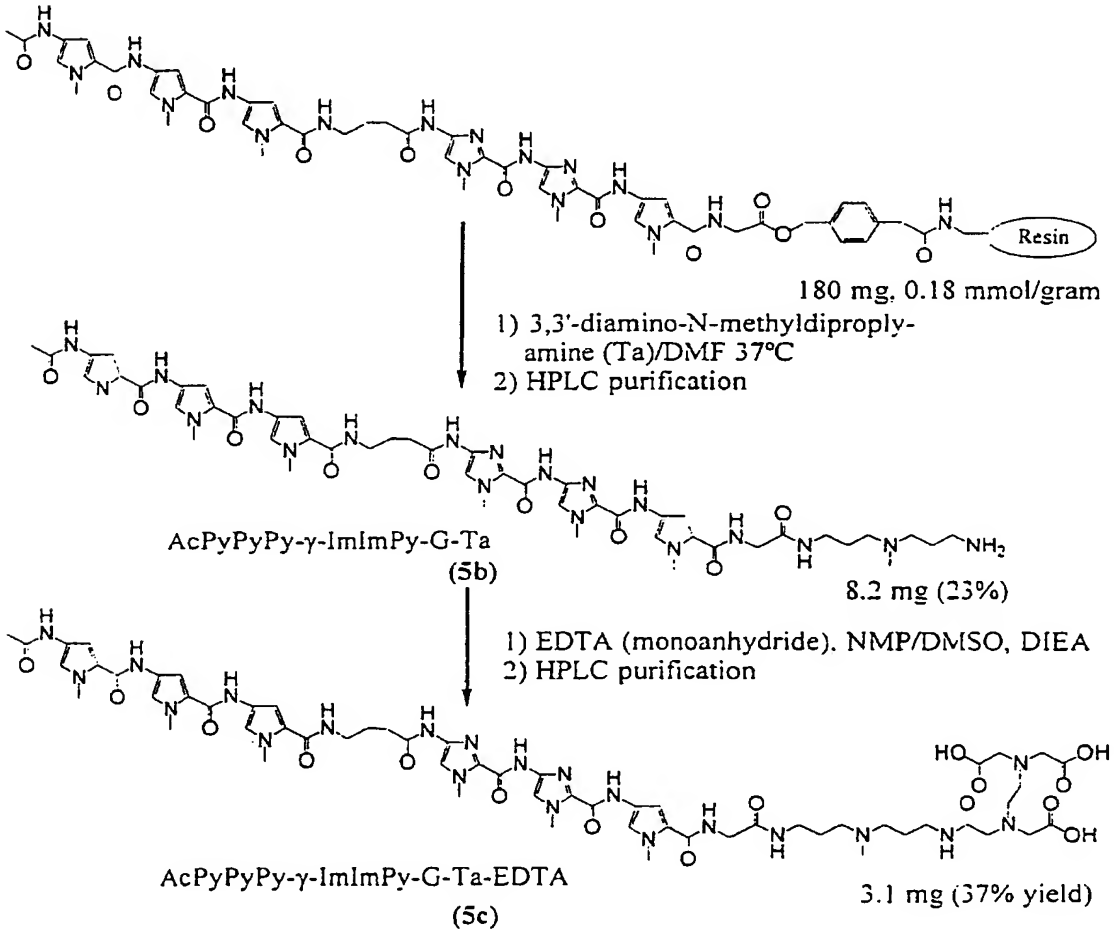
SCHEME 2



Synthesis of Polyamide Derivatives. The methods for the synthesis of minor-groove polyamides can be readily extended to the synthesis of various

derivatives. Scheme 3 illustrates the synthetic scheme for introduction of EDTA into the C-terminus of minor groove polyamides by cleavage from the resin with a symmetrical triamine followed by reaction with EDTA monoanhydride. (*See* Example 4).

SCHEME 3



C-terminus EDTA derivatized polyamides are typically recovered in approximately 30% yield after HPLC purification.

Example 4 further describes the synthesis of C-terminus dimethylaminoproplyamine (Dp), ethylenediamine (ED), 3,3'-diamino-N-methylpropylamine (Ta) and β-alanine (β) derivatized polyamides. Finally, Example 4 also describes the synthesis of N-terminus EDTA derivatized polyamides using the synthesis of EDTA-γ-ImPyPy-β-PyPyPy-G-Dp as an example. Briefly, the polyamide H_2N-γ-ImPyPy-β-PyPyPy-G-Dp is prepared by cleavage of H_2N-γ-ImPyPy-β-PyPyPy-G-Resin with dimethylaminopropylamine (Dp). The primary amine is then derivatized with EDTA as described in Scheme III and isolated by preparatory HPLC.

The intermediates, containing a free primary amine, provide access to a wide number of modified minor-groove polyamides. including, but not limited to intercalators, polysaccharide conjugates, photoreactive agents and metal chelates. Furthermore, a polyamide containing a free primary amine can be reacted with an activated carboxylic acid to synthesize polyamides of increasing complexity, thiol modified polyamides, or bromoacetic acid modified polyamides. Amine modified polyamides are also useful for attachment to an appropriate support for making affinity chromatography columns. The synthetic methods outlined for Boc-protected monomers substituted at the N-methyl group, allows for the synthesis of amino modified pyrrole monomers for the addition of EDTA into any region of the polyamide.

Effect of C-terminal Glycine and C-terminal β-alanine on DNA Binding Properties: Specifically for "Slipped" versus "Overlapped" Binding Modes.

The DNA-binding affinities of several polyamides having the core sequence ImPyPy-X-PyPyPy (X = G, β, γ, Py) to the targeted 13 bp site

5'-AAAAAGACAAAAA-3' (SEQ ID NO:2), and to the targeted 9 bp site 5'-TGTTAAACA-3' (SEQ ID NO:4) were examined by DNase I footprint titration (Galas and Schmitz (1978) Nucleic Acids Res. 5:3157-3170; Fox and Waring (1984) Nucleic Acids Res. 12:9271-9285; Brenowitz *et al.*(1986) Methods Enzymol. 130:132-181; Brenowitz *et al.* (1986) Proc. Natl. Acad Sci. U.S.A. 83:8462-8466; Senear *et al.* (1986) Biochemistry 25:7344-7354) (Example 10, Figure 15). The polyamide:DNA complexes predicted for the 13 bp and 9 bp target sites represent two distinct binding modes, referred to as "slipped" and "overlapped" (*see* Figure 11). The "slipped" (13 bp) binding mode (Figure 11A) integrates the 2:1 and 1:1 binding motifs at a single site. In this binding mode, the ImPyPy portion of two ImPyPy-X-PyPyPy polyamides bind the central 5'-AGACA-3' sequence in a 2:1 manner and the PyPyPy portion of the polyamides bind the A/T flanking sequences similar to the 1:1 complexes of Distamycin. In the "overlapped" (9 bp) binding mode (Figure 11B). two ImPyPy-X-PyPyPy polyamides bind directly opposite one another, with the ImPyPy portion of one polyamide opposite the PyPyPy portion of the other polyamide recognizing the 5 bp subsides 5'-TGTTA-3' and 5'-AAACA-3' as in the ImPyPy-Dp/Distamycin (PyPyPy) heterodimer.

In the 13 bp "slipped" and 9 bp "overlapped" sites described above. the GC and CG base-pairs are separated by one and five A/T base-pairs, respectively. It should be noted that "partially slipped" sites of 10, 11 and 12 bp in which the GC and CG base-pairs are separated by two. three and four A/T base-pairs, respectively, are also potential binding sites of the polyamides studied here.

Affinities for polyamides differing only in the presence or absence of a C-terminal glycine residue reveals that C-terminal glycine

dramatically affects the DNA-binding properties of polyamides. (*See* Table 3, Figure 12.) Relative to the polyamide ImPyPy-G-PyPyPy-Dp, which binds the "slipped" site 5'-AAAAAGACAAAAA-3' (SEQ ID NO:2) and the "overlapped" site 5'-TGTTAAACA-3' (SEQ ID NO:4) (with similar affinities (approximately 1 x 10^8 M^{-1}) (Figure 12A), polyamide ImPyPy-G-PyPyPy-G-Dp, which was previously prepared by solution methods and has a C-terminal glycine, binds these sites with approximately 1.5-fold (7 x 10^7 M^{-1}) and 80-fold (1.7 x 10^6 M^{-1}) lower affinities, respectively (Figure 12B). Also, relative to ImPyPy-G-PyPyPy-Dp, ImPyPy-G-PyPyPy-G-Dp binds to the 11 bp site 5'-TGTGCTGCAAG-3' (SEQ ID NO:6) with > 50-fold lower affinity (Figures 12A and 12B). Each data point is the average value obtained from three quantitative footprint titration experiments (Example 10). Similarly, relative to ImPyPy-β-PyPyPy-Dp, ImPyPy-β-PyPyPy-G-Dp binds 5'-AAAAAGACAAAAA-3' (SEQ ID NO:2) and 5'-TGTTAAACA-3' (SEQ ID NO:4) with approximately 1.5-fold and 10-fold lower affinities, respectively (Figures 12C and 12D). In both cases, C-terminal glycine confers specificity for "slipped" relative to "overlapped" complexes. In the case of ImPyPyPyPyPyPy-Dp and ImPyPyPyPyPyPy-G-Dp, the presence of a C-terminal glycine reduces the binding affinities at both the "slipped" and "overlapped" sites by factors of approximately 8 and 15, respectively (Figures 12G and 12H).

In contrast to ImPyPy-G-PyPyPy-Dp, ImPyPy-G-PyPyPy-β-Dp has DNA-binding affinities and specificities similar to ImPyPy-G-PyPyPy-Dp (Table 3, Figure 12F). Modeling indicates that ImPyPy-G-PyPyPy-Dp and ImPyPy-G-PyPyPy-β-Dp have similar DNA-binding surfaces at the C-terminal end of the polyamides (Figure 13). The disruption of "overlapped" 2:1

complexes by C-terminal glycine may result from a steric interaction between the glycine carbonyl group and the floor of the minor groove. In the "slipped" binding mode, the C-terminal part of the molecule is bound in a 1:1 manner, which is tolerant of C-terminal glycine (Figure 14).

Rates and Efficiency of Coupling Reactions. Under the standard coupling conditions the efficiency of coupling reactions is as follows, (activated ester/free amine), Py/G ≈ Im/G > G/Py ≈ G/Im > Im/Py > Py/Py > Im/Im > Py/Im. All couplings except for Im/Im and Py/Im are >99.8% complete in 42 minutes. The faster couplings are more than 99.8% complete within 5 minutes. For the Im/Im and Py/Im couplings, extended reaction times are recommended in order to assure complete reaction. Fortunately, these couplings occur least frequently in the current synthesis of the minor groove polyamides. The Py/Py coupling is the most common and was used as the model around which the synthetic methodology was optimized. Coupling rates are estimated based on picric acid titration data and ninhydrin tests when possible. No correction was made for the change in substitution of the resin resulting from the addition of a monomer, because the effect is very small for the low substitution resins used for synthesis. The change in substitution during a specific coupling or for the entire synthesis can be calculated as

$$L_{new} = L_{old}/(1 + L_{old}(W_{new} - W_{old}) \times 10^{-3}) \quad (1)$$

where L is the mmol of amine per gram of resin, and W is the weight ($gmol^{-1}$) of the growing polyamide attached to the resin. The subscript *old*, indicates a parameter before the coupling reaction. *new* indicates a parameter referring to the resin after a coupling reaction.

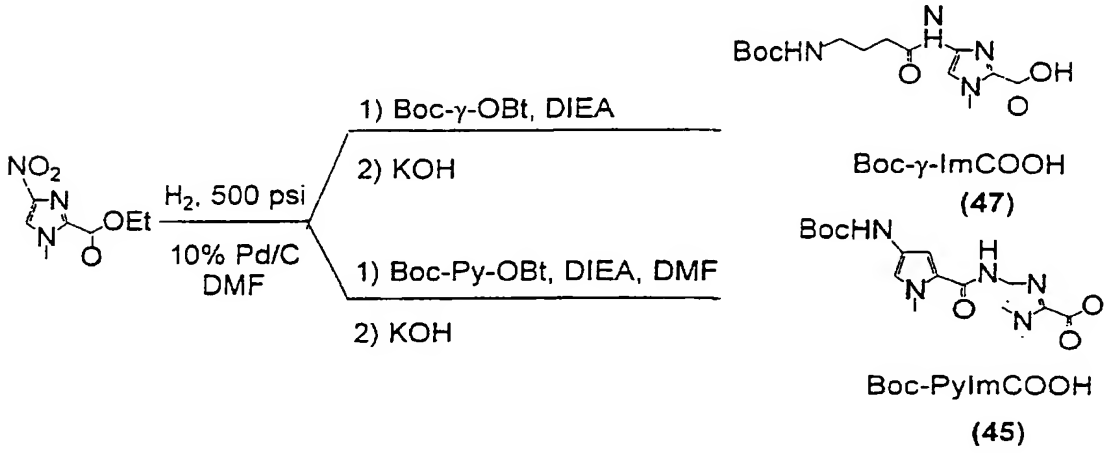
For the Py/Py, Im/Py, Py/G and G/Py couplings an attempt was made to measure rates using picric acid titration at 1 minute time intervals. The Im/Py, Py/G, and G/Py couplings all reached completion too rapidly to measure an accurate rate. For the Py/Py coupling, reasonably accurate data was obtained for monitoring the disappearance of amine. From the slope of a plot of ln (meq. amine) versus time, it is possible to estimate a rate of reaction of $0.18h^{-1}$ which corresponds to a 3.9 minute half life, and indicates that 25.6 minutes are required for 99% reaction, and 38.4 minutes for 99.9% reaction (Figure 16). A 45 minute coupling time was chosen to ensure complete reaction.

Preparation of Dimeric Building Blocks. As discussed above the amine group of imidazole is less reactive than the amine group of pyrrole. When coupling aliphatic amino acids to an imidazole amine, extended coupling times or double coupling is sometimes required. For the coupling of pyrrole to imidazole, a symmetrical anhydride protocol, in which pyrrole is activated by formation of the symmetrical anhydride in the presence of DMAP (Example 5) was developed. The reaction of the activated imidazole acid is extremely rapid, with dilute solutions (<0.1 M) reacting to completion in the standard coupling time when coupling is imidazole amine, pyrrole amine and aliphatic amines. To avoid the reduced reactivity of the imidazole amine, while taking advantage of the increased reactivity of the imidazole acid a set of dimeric building blocks were prepared.

The dimeric building blocks were prepared with a series of reactions analogous to the preparation of the Boc-imidazole monomer and require no flash chromatography. Scheme 4 (Example 11) illustrates this general method with the synthesis of the Boc-γ-ImCOOH (47) and Boc-

PyImCOOH (45) dimers. Both dimers can be prepared in multigram quantities without chromatography. The Boc- group is introduced to the imidazole amine with a Boc- protected -OBt activated amino acid. The resulting Boc-amino acid-imidazole-ethyl ester is isolated by precipitation from water, the ethyl group removed by alkaline hydrolysis, and the dimer is collected by filtration after acidification of the reaction mixture.

SCHEME 4



Solid Phase Synthesis of Cyclic Polyamides. Cyclic polyamides have also been found to bind to DNA. For example, the cyclic polyamide *cyclo*-(ImPyPy-γ-PyPyPy-γ-), which took more than a year to synthesize by solution phase methods, has been shown to bind the predicted target site 5'-WGWWW-3' (W = A or T) with high affinity and moderate specificity. (Cho *et al.* (1995) Proc. Natl. Acad. Sci. USA 92:10389). The outlined methods for the synthesis of straight chain polyamides are readily extendible to the

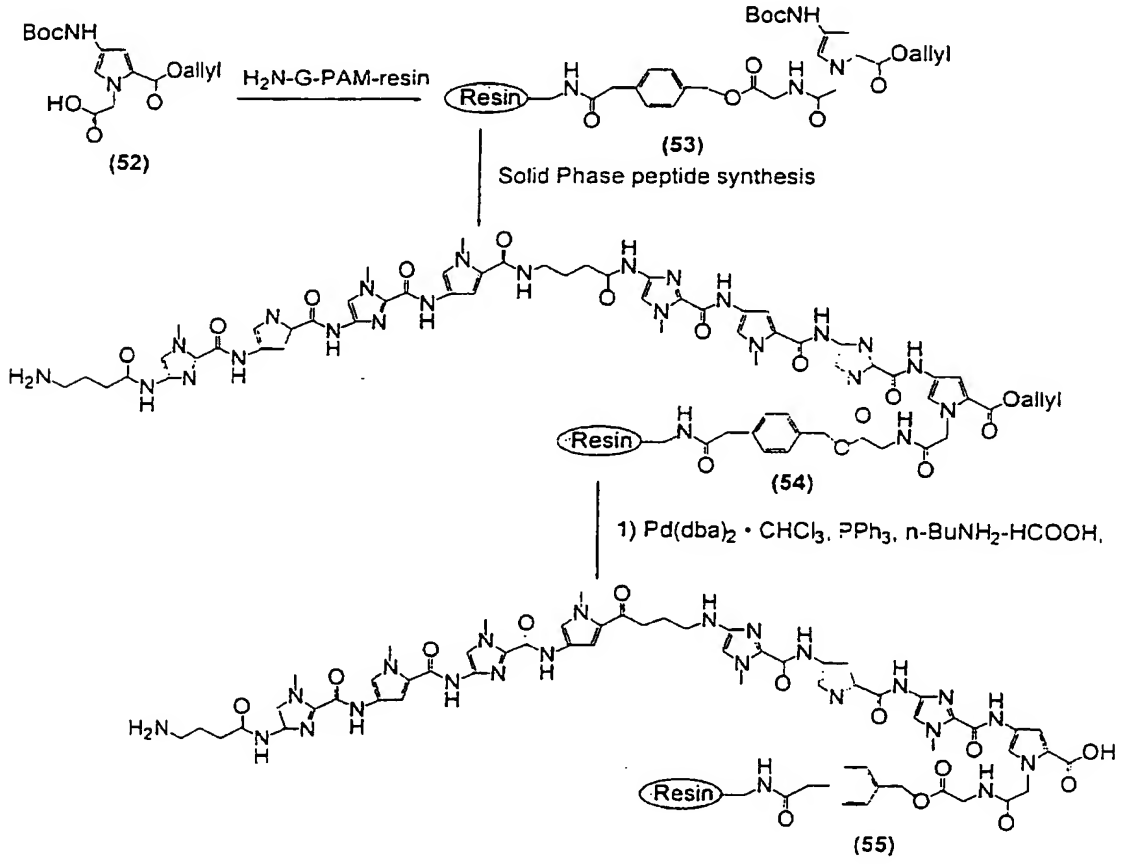
synthesis of cyclic polyamides. Using the solid phase peptide synthesis method of this invention cyclic polyamides can be prepared in large quantities in a matter of days. A typical synthetic scheme is outlined in Scheme 5 (*see* Examples 12 and 13).

A key intermediate for the solid phase synthesis of cyclic polyamides is the Boc-protected allyl ester pyrrole monomer (**52**), in which the N-methyl group is substituted so as to allow attachment to the resin. The synthesis of this monomer is described in Example 12 (Scheme 19). Briefly, methyl 4-nitropyrrole-2-carboxylate (**49**) (Fanta (1966) Org. Syn. Coll. 4:844; Morgan and Morrey (1966) Tetrahedron 22:57) is reacted with benzyl-2-bromoacetate in the presence of potassium iodide and anhydrous potassium carbonate to give the nitro-diester (**50**) in 85% yield. The nitro group is reduced to the amine and the benzyl ester simultaneously reduced to the acid with Pd/C catalyst and H_2. The amine is immediately protected with boc-anhydride and purified by flash chromatography to give the Boc-protected monoacid (**51**). The methyl ester is then reacted with allyl alkoxide to give the desired monomer (**52**). The allyl group is stable to both Boc- or Fmoc- chemistry, but is easily removed on the solid support with a soluble palladium catalyst to which the benzyl ester resin linkage is stable.

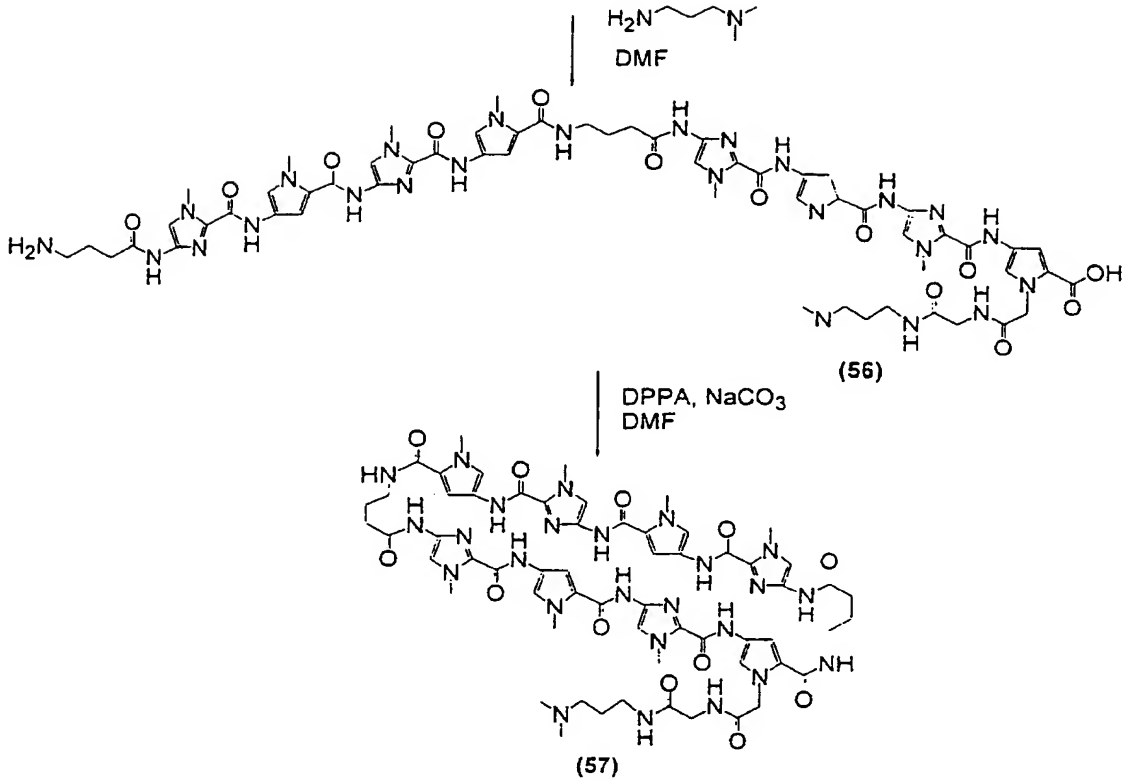
Referring back to Scheme 5, a single equivalent of the Boc-protected allyl ester pyrrole monomer (**52**), is attached to the Glycine-PAM-resin, to provide Boc-Py(O-allyl)-G-PAM-resin (**53**), in high yield. Standard manual solid phase methods, as described above, are then used to assemble the polyamide, H_2N-γ-ImPyImPy-γ-ImPyImPy(O-allyl)-G-PAM-resin (**54**). The allyl group is removed with a soluble palladium catalyst to yield acid (**55**), which is cleaved from the resin with dimethylaminopropylamine. HPLC

purification yields 105 mg of polyamide from cleavage of 0.25 mmol resin, a 45% yield. A small sample of precursor was then cyclized by treatment with diphenylphosphoryl azide (DPPA) in dilute DMF solution to yield the cyclized polyamide (57), which was purified by HPLC to yield 38% of the cyclic polyamide. Illustrative polyamides prepared by this method are set forth in Table 4.

SCHEME 5



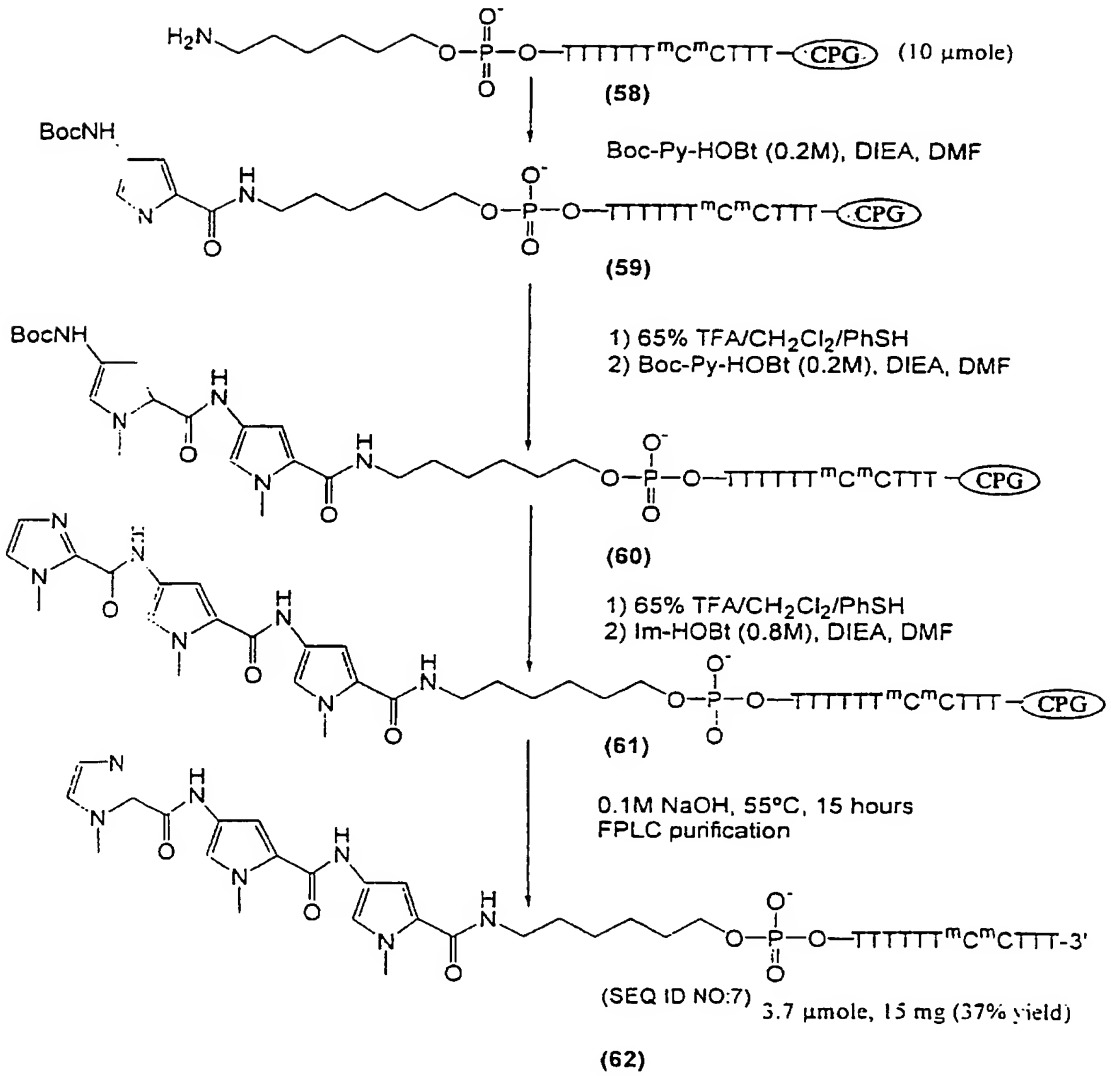
SCHEME 5 CON'T.



Synthesis of oligonucleotide-minor groove polyamide conjugates.

The methods for the synthesis of minor-groove polyamides are also readily extendable to the synthesis of oligonucleotide-minor groove polyamide conjugates. A typical synthesis of an oligonucleotide minor-groove polyamide is outlined in Scheme 6 (*see* Example 13).

SCHEME 6



The oligonucleotide portion of the molecule (10 μmol) was assembled on an automated DNA synthesizer using standard DNA cycles. a commercially available 5'-MMT-C6-amino modifier (MMT = monomethoxytrityl) was attached using an extended 10 minute synthesis cycle. (Connolly and Rider

(1985) Nuc. Acid. Res. 13:4485; Sproat *et al.* (1987) Nuc. Acid. Res. 15:4837; Juby *et al.* (1991) Tet. Lett. 32:879-882). The MMT group was removed from the modified oligonucleotide by manual treatment with 3% trichloroacetic acid (TCA) in dichloromethane. The controlled pore glass support was then removed from the synthesis cartridge and transferred to a standard peptide synthesis reaction vessel. The oligonucleotide (**58**) was reacted with a 0.2 M solution of Boc-Py-OBt in DMF/0.4M DIEA for 45 minutes. The reaction was determined to be complete by the quantitative ninhydrin test. which showed a distinct blue color for the oligonucleotide-polyamide conjugate (**59**), consistent with a 0.05 mmol/gram loading, and a lack of a blue color after 1 hour of reaction time. The Boc- group was removed with 65% TFA CH_2Cl_2/0.5M PhSH for 20 minutes and a second Boc-protected pyrrole coupled to form the aromatic carboxamide (**60**). The Boc- group is removed with TFA and the polyamide capped with N-methylimidazole-2-carboxylic acid. The oligonucleotide-polyamide conjugate (**61**)was then simultaneously cleaved from the resin and deprotected by treatment with 0.1M NaOH at 55°C for 15 hours and purified by FPLC chromatography.

A single reversed phase purification yields the polyamide conjugate, ImPyPy-$CONH(CH_2)_6$-$P(O)_4$TTTTTT^{m}C^{m}CTTT-3' (**62**)(SEQ ID NO:7) (^{m}C = methylcytidine), in high purity. The product obtained is characterized by a number of techniques (data not shown). MALDI-time of flight mass spectroscopy shows a single peak corresponding to a molecular mass of 3813.5 (predicted mass of 3814.3), indicating that full length product has been isolated. Reverse phase HPLC analysis of 10 nmoles of the conjugate, exhibits one major product, absorbing at both oligonucleotide wavelength (260) and polyamide wavelength (340). Enzymatic digestion and

subsequent HPLC analysis of a 10 nmole sample of conjugate is consistent with the proposed composition of the oligonucleotide.

Ultraviolet spectroscopy indicates an additive spectra as might be expected for a conjugate of 2-imidazole Netropsin and an 11-mer thymidine rich oligonucleotide. From the extinction coefficient of the bases. 8,800 for thymidine (T) at 260 nm and 5,700 for methylcytidine (MeC) at 260 nm and the reported extinction coefficients for 2-ImN of 19,000 (255 nm) and 26,000 (302 nm), it is possible to predict the ratio of the extinction coefficients at 260 nm. (Colocci *et al.* (1993) J. Am. Chem. Soc. 115:4468-4473). Assuming a contribution from the oligo of 90,600 and from the polyamide of 19,000, a ratio of 4.2 is expected and a ratio of 3.7 is observed.

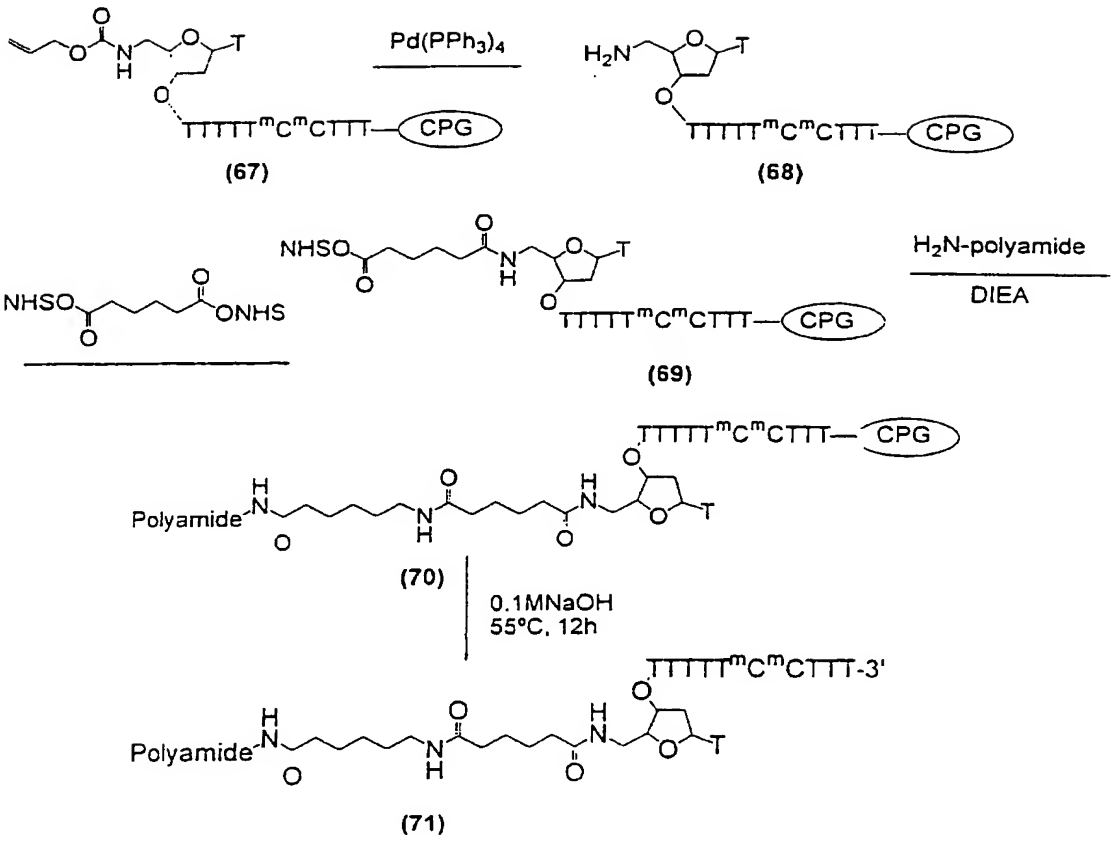
Finally, a 14 mg sample of the polyamide conjugate was dissolved in 700 µl of deuterium oxide and analyzed by ^{1}H NMR spectroscopy at 300 MHz. Although most of the spectrum is complex, the aromatic region is readily interpreted. The protons expected in the aromatic region correspond to the polyamide ring protons, and the C_6 ring protons of thymidine and 5-methylcytidine. The observed spectrum is consistent with the predicted sequence ImPyPy-CONH$(CH_2)_6$-P(O)$_4$TTTTTT^{m}C^{m}CTTT-3', with the 2 protons observed at 7.7 corresponding to the cytidine, 9 protons at 7.6 corresponding to the thymidine contribution, 2 protons at 7.3 corresponding to the imidazole ring, four pyrrole doublets at 7.2, 7.1, 6.9, and 6.7 corresponding to four protons, and 11 anomeric protons at 6.2. The purity of the sample, as determined by NMR, is > 98%. The ability to rapidly obtain NMR data (30 minutes of acquisition) on a molecule of this size (3 kD) warrants a synthesis scale such as the one chosen here. Table 5 sets forth illustrative oligonucleotide-polyamide conjugates synthesized by the method of this

invention.

In another embodiment (Scheme 7), the oligonucleotide, prepared using standard phosphoramidite chemistry, is capped with a 2', 5'-dideoxy-5'-aminothymidine (Smith *et al.* (1985) Nucleic Acids Research 13:2399), prior to attachment of the polyamide (**68**).

The free amino group of the oligonucleotide is then reacted with the bis NHS ester of glutaric acid (DMF/DIEA) for 2 hours at room temperature to form activated acid (**69**). Excess NHS ester is removed by washing with a large excess of DMF. The activated acid is then treated with an equivalent of a polyamide containing a free amine prepared by the method of this invention. The coupling reaction (DMF/DIEA) is allowed to proceed for 12 hours, and any unreacted polyamide is removed by washing the resin. The oligonucleotide (**70**) is deprotected and simultaneously cleaved from the resin with a solution of 0.1 M NaOH at 55°C for 12 hours. The polyamide-oligonucleotide conjugate (**71**) is then purified by a single reverse phase chromatography step (C18, TEAA, pH 7), to give a 10% yield. A list of illustrative polyamide-oligonucleotide conjugates which have been prepared by the method of this invention is set forth in Figure 18 and Table 5. Figure 19 depicts a ribbon graphic illustrating how the conjugate Dp-G-PyPyPy-G-PyPyIm-linker-TTTTTT^{m}C^{m}CTTT-3' might bind to the double helical DNA.

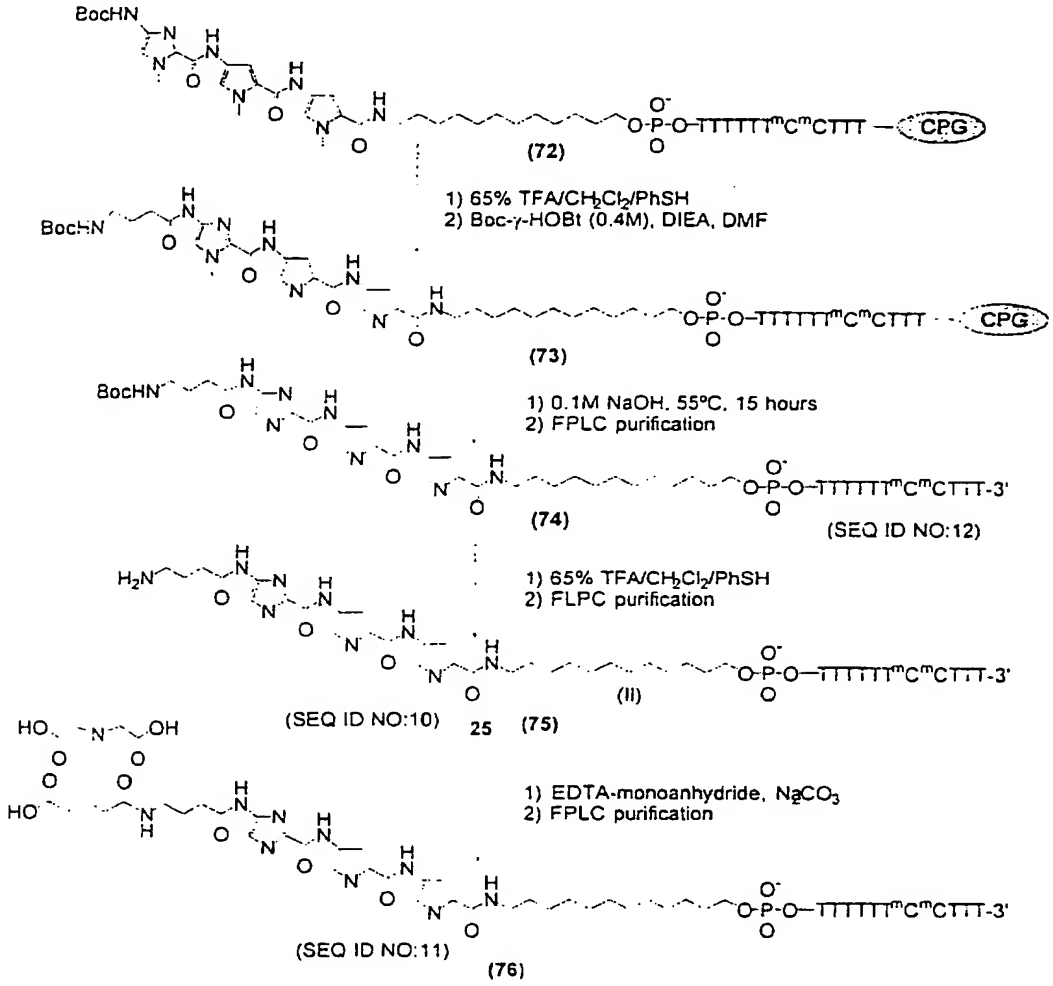
SCHEME 7



Preparation of modified derivatives. In yet another embodiment of this invention, modified polyamide-oligonucleotide conjugates having terminal 1°, 2° and 3° amino groups are prepared. Modifications include, but are not limited to the formation of the dimethylaminopropylamine (Dp), the γ-aminobutyric acid (γ), the ethylenediamine (ED), the 3, 3'-diamino-N-methylpropylamine (Ta) or the ethylenediaminetetraacetic acid (EDTA) derivatives.

Scheme 7 illustrates this method by the formation of the γ-aminobutyric derivative (75) which is then further modified by formation of the EDTA derivative (76).

SCHEME 8



Referring to Scheme 7, the conjugate Boc-ImPyPy-CONH$(CH_2)_{12}$-P(O)$_4$-TTTTTT^{m}C^{m}CTTT-CPG (72) is synthesized on solid support as described above. The Boc- group is removed with TFA under standard conditions and the product is reacted with the HOBt ester of Boc-γ (generated *in situ*). The

product, Boc-γ-ImPyPy-CONH$(CH_2)_{12}$-P(O)$_4$-TTTTTT^{m}C^{m}CTTT-CPG (73) is simultaneously cleaved from the resin and the bases deprotected by treatment with 0.1M NaOH at 55°C for 15 hours. The mixture is then purified by FPLC to give Boc-γ-ImPyPy-CONH$(CH_2)_{12}$-P(O)$_4$-TTTTTT^{m}C^{m}CTTT-3' (SEQ ID NO:12) (74) which is characterized by HPLC, enzymatic degradation and mass spectroscopy. The Boc- group is then removed by treatment with TFA under standard conditions and the mixture purified by FPLC to give H_2N-γ-ImPyPy-CONH$(CH_2)_{12}$-P(O)$_4$-TTTTTT^{m}C^{m}CTTT-3' (75) (SEQ ID NO:10) which is characterized by mass spectroscopy. The oligonucleotide-polyamide conjugate is then reacted with the monoanhydride of EDTA in pH 9.5 carbonate buffer to yield the EDTA derivative (76) (SEQ ID NO:11).

Synthesis of protein-minor groove polyamide conjugates.

In another embodiment of this invention the method of preparing polyamides is extended to the preparation of polyamides that are attached to a protein (referred to herein as a protein-polyamide conjugate). This method is illustrated by the replacement of the DNA binding domain of the major groove DNA binding protein GCN-4 (Oakley and Dervan (1989) Science 248:847) with the polyamide. NH_2-β-β-ImPyPy-γ-PyPyPy-γ-γ-, prepared as described above. A ribbon model of GCN-4 is depicted in Figure 20. As illustrated in Figure 20 the first 50 residues comprise an α- helix which has a DNA binding domain and a coiled coil dimerization region. The coiled-coil region --Lys Gln Leu Glu Asp Lys Val Glu Glu Leu Leu Ser Lys Asn Tyr His Leu Glu Asn Glu Val Ala Arg Leu Lys Lys Leu Val Gly Glu Arg-CO_2NH_2 (SEQ ID NO:24)-- is prepared using standard NMP/HOBt methods for an ABI 430A peptide synthesizer. The polyamide domain is then appended in a stepwise fashion using the synthetic methods described above. The conjugate H_2N-β-β-

ImPyPy-γ-γ- protein (SEQ ID NO:25) is then cleaved from the resin with anhydrous HF, and purified by reversed phase HPLC chromatography, to provide 1.1 mg of conjugate from cleavage of 100 mg of resin. The conjugate has been characterized by analytic HPLC and mass spectroscopy. Figure 21 illustrates the DNA binding domain of the GCN-4- polyamide conjugate.

The ability to easily prepare polyamides with an appended peptide moiety is useful, since peptide leader sequences often provide a means for delivering molecules into cells. (Soukchareun (1995) J. Bioconj Chem. 6.1:43-53)

Automated synthesis of Polyamides.

The methods of this invention for the syntheses of polyamides are suitable for automation. A peptide synthesizer 110, shown in Figure 22, was modified to prepare polyamides containing imidazole and pyrrole carboxamides. The peptide synthesizer 110 has three chemistry centers where the reactions occur: an activator center 112, a concentrator center 114 and a reaction center 118. The activator center (first chemistry center) 112 is not used in preparing the polyamides. The concentrator center (second chemistry center) 114 is coupled to the activator center 112 by a tube 116. The reaction center (third chemistry center) 118 is coupled to the concentrator center 114 by a tube 120. A first valve 122 controls the flow of the contents from the activator center 112 to the concentrator center 114. A second valve 123 controls the flow of the contents from the concentrator center 114 to the reaction center 118. Both valves 122, 123 are coupled to a controller 124, that provides signals that control both valves 122, 123.

Another valve 125, operated by the controller 124, connects the reaction center 118 to a drain 126. A shaker 127, operated by the controller 124, shakes the reaction center 118. The three chemistry centers 112, 114 and 118 are coupled to a plurality of reagents by a valve matrix 128. The reagents are contained in a plurality of bottles 130 in one of ten reagent positions. The valve matrix allows any of the reagents in reagent positions 1-10 to flow into any of the three chemistry center 112, 114, 118. The valve matrix has programmable valves that are controlled by the controller 124. The pressurized air source 32 allows aeration of each of the three chemistry center 112, 114 and 118.

A plurality of cartridges 134 typically contains predissolved/preactivated monomer units. A programmable needle 36 transfers the contents of the cartridges 134 to one of the plurality of chemistry center 112, 114 and 118. The programmable needle 136 is directed by the controller 124.

The controller 124 is connected to a floppy disk drive 138. The floppy disk drive 138 accepts a floppy disk 140 having a storage medium encoded with a computer program to direct the operation of the peptide synthesizer 110. Figure 23 is a flow chart of a computer program used to produce polyamides containing imidazole and pyrrole carboxamides in the peptide synthesizer 110. The process begins at block 200. First, preactivated monomer is dissolved in DMF and transferred from one of the plurality of cartridges 134 to the concentrator 114, at block 202. bypassing the activator center 112. DIEA, from one of the plurality of reagent bottles 130, is transferred to the concentrator 114, at block 204. Simultaneously, a resin in the reaction center 118 is treated with TFA and PhSH, at block 206. The TFA and

PhSH are contained in the reagent bottles 30. The resin is used to support the growing polyamide. Next, the TFA is drained for the reaction center 118, at block 208. Dichloromethane and TFA washes are performed before and after TFA/PhSH treatment. The dichloromethane and PhSH are contained in the plurality of reagent bottles 130. Following the dichloromethane/TFA wash, the contents of the concentrator 14 are transferred to the reaction center 118, at block 210. At block 212, a shaker starts shaking the reaction center 118 and a timer in the controller is started. At t=1800s DMSO from one of the plurality of reagent bottles is added to the reaction center and DIEA from one of the plurality of reagent bottles is added to the reaction center, at block 214. The reaction center 118 is then drained, at block 216. The reagent Ac_2O is added to the reaction center at block 218. The reaction center 118 is then drained again at block 220. At block 222, it is determined if a timer is greater than or equal to two hours. The process waits at block 222 until the timer equals or exceeds two hours. Then the shaking is stopped at block 224 and the process ends at block 226.

The machine assisted protocols are highly efficient as demonstrated by the synthesis of the 8 residue polyamide ImPyPy-γ-PyPyPy-β-Dp, with the crude reaction product containing > 70% of the desired polyamide as determined by HPLC analysis. It is possible, however, to modify other commercially available peptide synthesizers and organic synthesis machines to accommodate the automated chemistry performed to synthesize the polyamides of this invention.

The versatility of the machine assisted protocols is demonstrated by the synthesis of polyamides H_2N-β-PyPyPy-γ-ImImPy-β-β-β-β-PyPyPy-γ-ImImPy-β-Dp and H_2N-β-PyPyPy-γ-ImImPyPy-β-β-PyPyPyPy-γ-ImImPy-β-

Dp both of which were characterized by HPLC, ^{1}H NMR, and MALDI-TOF mass spectroscopy. Figure 24 depicts a ball and stick model of the projected binding mode of polyamide (#) with the target sequence 5'-TGGTTAGTACCT-3' (SEQ ID NO:5) on PXLO-wt. The binding affinity was determined to be approximately 1 x 10^9 M^{-1}. Introduction of a single base pair mismatch in the binding site lowers affinity by apprxomately a factor of 10-fold.

EXAMPLES

Materials. Di-t-butyldicarbonate (Boc-anhydride), Boc-G-(-4-carboxamidomethyl)-benzyl-ester-copoly(styrene-divinylbenzene) resin [Boc-G-PAM-resin] (0.2 mmol/gram), dicyclohexylcarbodiimide (DCC), hydroxybenzotriazole (HOBt) and aminomethylated polystyrene resin (0.7 mmol/gram) were purchased from Peptides International (Louisville, Kentucky). *N,N*-diisopropylethylamine (DIEA), *N,N*-dimethylformamide (DMF), acetic anhydride, *N*-methylpyrrolidone (NMP), 0.0002M potassium cyanide/pyridine, dimethylsulfoxide (DMSO) and DMSO/NMP were purchased from Applied Biosystems. Boc-Glycine (Boc-G) was purchased from Peninsula, Boc-γ-aminobutyric acid (Boc-γ) from NOVA Biochem, dichloromethane from EM. thiophenol (PhSH) and picric acid from Aldrich, trifluoroacetic acid (TFA) from Halocarbon, phenol from Fisher, and ninhydrin from Pierce. Unless stated otherwise reagent-grade chemicals were used. Additionally, all reagents were used without further purification. Quik-Sep polypropylene disposable filters were purchased from Isolab Inc. and were used for filtration of dicyclohexylurea (DCU) for washing the resin for the

ninhydrin and picric acid tests. A shaker for manual solid phase synthesis was obtained from Milligen. Screw-cap glass peptide synthesis reaction vessels (5 ml) with a #2 scintered glass frit were made at the California Institute of Technology glass shop as described by Kent. (Kent (1988) Ann. Rev. Biochem. 57:957).

DNA Reagents and Materials. Sterilized 0.1% DEPC-treated water (Sambrock *et al.* (1989) Molecular Cloning, 2nd ed.; Cold Spring Harbor, New York) was either prepared or purchased from Gibco. Polyacrylamide gel electrophoresis was performed in a 1X TBE buffer. Autoradiography was carried out using Amersham Hyperfilm MP or Kodak X-Omat film. Gels were analyzed by storage phosphor technology. (Miyahara *et al.* (1986) Nuc. Inst. Meth. Phys. Res. A246:572-578: Johnston *et al.* (1990) Electrophoresis 11:355-360).

NMR were recorded on a GE 300 instrument operating at 300 MHz (^{1}H) and 75 MHz (^{13}C). Chemical shifts are reported in ppm relative to the solvent residual signal. UV spectra were measured on a Hewlett-Packard Model 8452A diode array spectrophotometer. Matrix-assisted, laser desorption/ionization time of flight mass spectrometry was carried out at the Protein and Peptide Microanalytical Facility at the California Institute of Technology. HPLC analysis was performed either on a HP 1090M analytical HPLC or a Beckman Gold system using a RAINEN C_{18}, Microsorb MV, 5 μm, 300 x 4.6 mm reversed phase column in 0.1% (wt/v) TFA with acetonitrile as eluent and a flow rate of 1.0 ml/min, gradient elution 1.25% acetonitrile/min. Preparatory HPLC was carried out on a Beckman HPLC using a Waters DeltaPak 25 x 100 mm, 100 μm C_{18} column equipped with a guard, 0.1% (wt/v) TFA, 0.25% acetonitrile/min. 18MΩ water was obtained from a

Millipore MilliQ water purification system, all buffers were 0.2 μm filtered. Flash column chromatography was carried out using Silica Gel 60 (230-400 mesh, Merck). Thin layer chromatography (TLC) was performed on Silica Gel 60 F_{254} precoated plates (Merck).

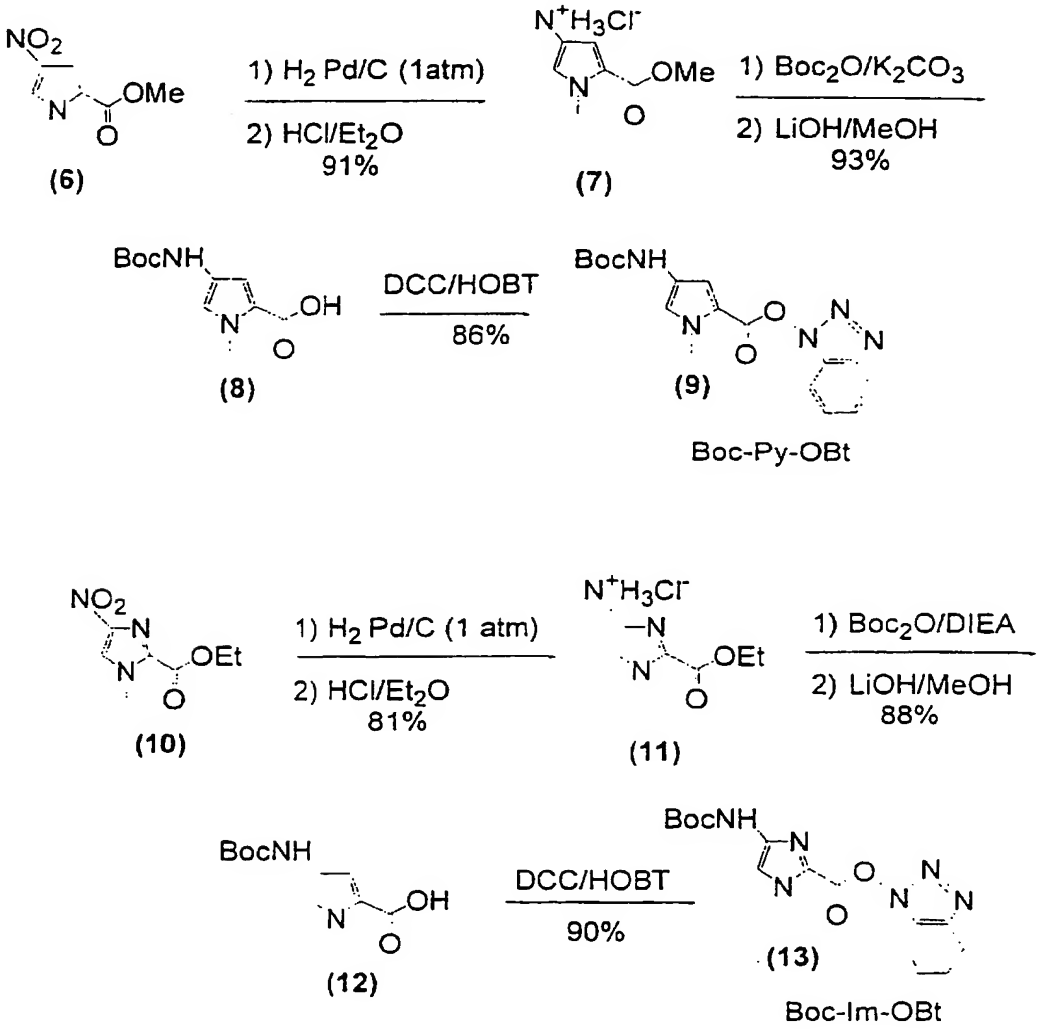
Example 1. Preparation of the Pyrrole and Imidazole Monomers

Preparation of Boc-Py-OBt (9) and Boc-Im-OBt (13).

The Boc-Py-OBt (**9**) and Boc-Im-OBt (**13**) monomers were synthesized starting from the known nitro-esters **6** and **10** (prepared as described in Schemes 10 and 11 (Bailey *et al.* Org. Syn.101-102: Corwin and Quattlebaum (1936) J. Am. Chem. Soc. 58:1081-1085; Grehn (1978) Chim. Scripta 13:67-77; Morrey and Morrey (1966) Tetrahedron 22:57-62; Krowicki and Lown (1987) J. Org. Chem. 52:3493-3501) as outlined in Scheme 8 below. Reduction of the nitro group gave amines **7** and **11**, in 91% and 81% yield respectively. The amines were Boc- protected with Boc-anhydride (pyrrole amine **7** in aqueous carbonate/dioxane and imidazole amine **11** in DMF/DIEA) and the ester groups were hydrolyzed with aqueous sodium hydroxide to yield the Boc- protected acids **8** and **12** in 93% and 88% yields, respectively. The acids were then activated at high concentration (>0.2 M acid in DMF) with 1 equivalent of DCC and HOBt and the -OBt esters precipitated from water to give compounds **9** and **13**. Overall yields starting from the nitro methyl esters are reproducibly greater than 60% for both the pyrrole and imidazole -OBt esters, with simple purification requiring no column chromatography. The Boc-imidazole acid has been reported to decarboxylate even at reduced temperature. The Boc-Im-OBt ester **13**, however, has been found to be stable

at room temperature, with HOBt effectively acting as a protecting group for the unstable imidazole carboxylic acid.

SCHEME 9



Preparation of Methyl 4-amino-1-methyl-pyrrole-2-carboxylate hydrochloride (7). Recrystallized methyl 1-methyl-4-nitropyrrole-2-carboxylate **6** (40 g, 0.22 mol) was dissolved in 900 ml ethyl acetate. A slurry

of 10 g of 10% Pd/C in 100 ml ethyl acetate was added and the mixture stirred under a slight positive pressure of hydrogen (about 1.1 ATM) for 48 hours. The Pd/C was removed by filtration through Celite, washed with 50 ml ethyl acetate, and the volume of the mixture was reduced to about 200 ml. 700 ml of ethyl ether was added and HCl gas gently bubbled through the mixture while maintaining a temperature below 20°C. The precipitated amine hydrochloride was then collected after storage at -20°C for 40 hours to yield (38 g, 91%) of a very white powder. TLC (ethyl acetate) Rf amine (0.6), Rf salt (0.0); ^{1}H NMR (DMSO-d_6) δ 10.23 (br s, 3H), 7.24 (d, 1H, J = 1.9 Hz), 6.79 (d, 1H, J = 2.0 Hz), 3.83 (s, 3H), 3.72 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 160.8, 124.3, 121.2, 113.4, 112.0, 51.8, 37.1.

Preparation of 4-[[(*tert*-Butyloxy)carbonyl]- amino]-1-methylpyrrole-2-carboxylic acid (8). The hydrochloride salt of the pyrrole amine 7 (24 g, 146 mmol) was dissolved in 300 ml of 10% aqueous sodium carbonate and di-t-butyldicarbonate (40 g, 174 mmol) slurried in 75 ml of dioxane was added over a period of ten minutes at room temperature. The reaction was allowed to proceed at room temperature for two hours and then cooled to 5°C for 2 hours. The resulting white precipitate was collected by vacuum filtration. The crude product was dissolved in 350 ml MeOH and 350 ml of 1M NaOH was added and the solution was heated at 60°C for 6 hours. The reaction was then cooled to room temperature, washed with ethyl ether (2 x 500 ml). The pH of the aqueous layer was reduced to approximately 3 with aqueous citric acid and was extracted with ethyl acetate (4 x 500 ml). The combined ethyl acetate extracts were dried with sodium sulfate and concentrated *in vacuo* to give a tan foam. The foam was dissolved in 100 ml of dichloromethane and 400 ml petroleum ether was added and the resulting

slurry was concentrated *in vacuo*. This step was repeated three times to give (31 g, 93% yield) of a fine white powder. TLC (7:2 benzene/ethyl acetate v/v) Rf (ester) 0.8, Rf (acid) 0.1. (ethyl acetate), Rf (acid) 0.6; ^{1}H NMR (DMSO-d_6) δ 12.10 (s, 1H), 9.05 (s, 1H), 7.02 (s, 1H), 6.55 (s, 1H), 3.75 (s, 3H), 1.41 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 162.4, 153.2, 123.3, 120.1, 119.2, 107.9, 78.9, 36.6, 28.7.

Preparation of 1,2,3-Benzotriazol-1-yl 4[[(*tert*-Butyloxy)carbonyl]-amino]-1-methylimidazole-2-carboxylate (9). The Boc-pyrrole-acid **8** (31 g, 129 mmol) was dissolved in 500 ml DMF and hydroxybenzotriazole (17.4 g, 129 mmol) was added followed by DCC (34 g, 129 mmol). The reaction was allowed to stir for 24 hours and then filtered *dropwise* into a well stirred solution of 5 liters of water (0°C). The precipitate was allowed to sit for 15 minutes at 0°C and then collected by filtration. The wet cake was dissolved in 500 ml of dichloromethane, washed with 200 ml brine, and added slowly to a stirred solution of petroleum ether at -20°C. After 4 hours at -20°C the precipitate was collected by vacuum filtration and dried *in vacuo* to give (39 g, 85% yield) of a finely divided white powder. (A yellowish impurity may be observed, which can be removed by flash chromatography (acetone:dichloromethane), followed by precipitation in petroleum ether). TLC (7:2 benzene/ ethyl acetate v/v) Rf 0.6; ^{1}H NMR (DMSO-d_6) δ 9.43 (s, 1H), 8.12 (d, 1H, J = 8.4 Hz), 7.80 (d, 1H, J = 8.2 Hz), 7.64 (t, 1H, J = 7.0 Hz), 7.51 (m, 2H), 7.18 (s, 1H), 3.83 (s, 3H), 1.45 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 156.5, 153.3, 143.2, 129.6, 129.2, 125.7, 125.2, 124.6, 120.3, 112.8, 110.3, 109.8, 79.5, 36.8, 28.6.

Preparation of Ethyl 4-amino-1-methylimidazole-2-carboxylate hydrochloride (11). Nitro imidazole ethyl ester **10** (10 g, 50 mmol) was

dissolved in 500 ml of 1:1 ethanol/ethyl acetate, 1 g 10% Pd/C slurried in 50 ml ethyl acetate was added and the mixture was stirred under a slight positive pressure of hydrogen (approximately 1.1 atm) for 48 hours. The reaction mixture was filtered, concentrated *in vacuo* and dissolved in 600 ml ether. HCl gas was bubbled through the ether solution at 0°C to give a white precipitate. The solution was cooled at -20°C for 4 hours and the precipitate was collected by vacuum filtration and dried *in vacuo* to give (8.1 g, 81% yield) of **11** as a fine white powder. ^{1}H NMR (DMSO-d_6) δ 10.11 (br s, 3H), 7.43 (s, 1H), 4.28 (q, 2H, J = 7.1 Hz), 3.92 (s, 1H), 1.28 (t, 3H, J = 7.1 Hz); ^{13}C NMR (DMSO-d_6) δ 157.6, 132.6, 117.4, 117.3, 61.8, 36.6, 14.5.

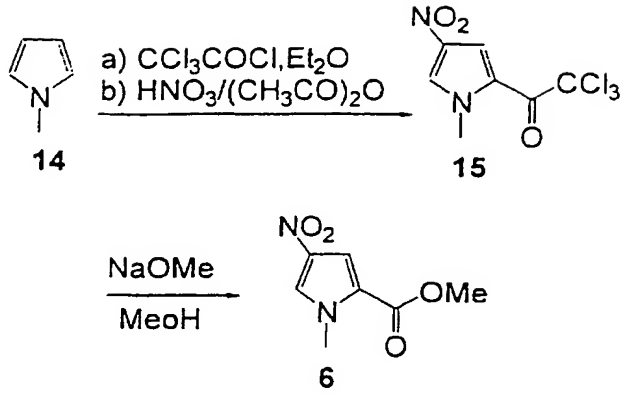
Preparation of 4-[[(*tert*-Butyloxy)carbonyl]- amino]-1-methylimidazole-2-carboxylic acid (**12**). The imidazole amine **11** (3 g, 14.5 mmol) was dissolved in 20 ml DMF and diisopropylethylamine (4.5 ml, 25 mmol) was added followed by di-t-butyldicarbonate (6 g, 27 mmol). The mixture was shaken at 40°C for 18 hours, allowed to return to room temperature and then partitioned between 500 ml of brine and 500 ml of ethyl ether. The ether layer was extracted with (2 x 200 ml each) 10% citric acid, brine, saturated sodium bicarbonate and brine. The ether layer was dried over sodium sulfate and concentrated *in vacuo* to yield the Boc-ester. The crude Boc-ester was dissolved in 40 ml of MeOH and 40 ml of 1 M KOH was added. The reaction mixture was shaken at 40°C for 4 hours, cooled to room temperature and partitioned between 200 ml of water and 300 ml ethyl ether. The aqueous layer was washed with 300 ml ethyl ether, the ether washes were discarded, and the pH of the aqueous layer was brought down to approximately 3 with 10% aqueous sodium bisulfate. The aqueous layer was extracted (10 x 150 ml) with ethyl acetate and the organic layers were combined, dried over

sodium sulfate and concentrated *in vacuo* to yield pure **12** as a white chalky powder (3.1 g, 88% yield). ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H), 7.23 (s, 1H), 3.85 (s, 3H), 1.41 (s, 9H).

Preparation of 1,2,3-Benzotriazol-1-yl 4[[(*tert*-butyloxy)carbonyl]-amino]-1-methylpyrrole-2-carboxylate (**13**). The Boc-imidazole-acid **12** (2 g, 8.3 mmol) was in dissolved in 10 ml of DMF and 1-hydroxybenzotriazole was added (1.2 g, 9 mmol) followed by DCC (2.4 g, 9 mmol). After 6 hours the precipitate was removed by filtration and washed with 4 ml of DMF. The DMF solution was added *dropwise* to 250 ml of well stirred ice water and the resulting precipitate was collected by vacuum filtration. The filter cake was ground and dried *in vacuo* over P_2O_5 to give (2.7 g, 89%) of **13** as a pale yellow power contaminated with a small amount of DCU (2.7 g, 89%). ^{1}H NMR (DMSO-d_6) δ 9.62 (s, 1H), 7.96 (s, 1H), 7.68 (d, 1H), 7.52 (d, 1H). 7.38 (d, 1H), 7.23 (s, 1H), 3.85 (s, 3H), 1.33 (s, 9H).

Preparation of Methyl 1-methyl-4-nitropyrrole-2-carboxylate (**6**) and Ethyl 1-methyl-4-nitroimidazole-2-carboxylate (**10**). Nitroesters **6** and **10** were synthesized from the inexpensive N-methylpyrrole and N-methylimidazole as outlined in Schemes 10 and 11, respectively. Each of these compounds can be prepared economically on a large scale. Methyl 1-methyl-4-nitropyrrole-2-carboxylate (**6**) was prepared using a modification of the reported synthesis of pyrrole-2-trichloroketone. (Bailey *et al.* (1971) Org. Syn. 51:101). Briefly, reaction of the inexpensive N-methylpyrrole (**14**) with trichloroacetylchloride followed by nitration with nitric acid gave nitropyrrole trichloroketone (**15**), which was treated with sodium methoxide to yield nitropyrrole (**6**).

SCHEME 10



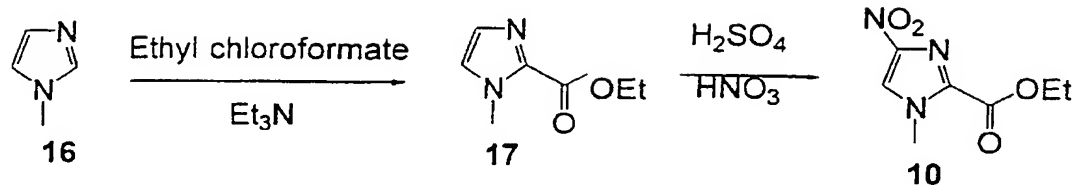
Preparation of 4-nitro-2-trichloroacetyl-1-methylpyrrole (15). To a well stirred solution of trichloroacetylchloride (1 kg, 5.5 mole) in 1.5 liters of ethyl ether was added dropwise over a period of 3 hours a solution of N-methylpyrrole (14) (0.45 kg, 5.5 mole) in 1.5 liters of anhydrous ethyl ether. The reaction was allowed to stir for an additional 3 hours and quenched by the dropwise addition of a solution of 400 g of potassium carbonate in 1.5 liters of water. The layers were separated and the ether layer was concentrated *in vacuo* to provide 2-trichloroacetyl pyrrole (1.2 kg, 5.1 mole) as a yellow crystalline solid, which can be purified by recrystallization from benzene, but is sufficiently pure to be used without further purification. To a cooled (-40°C) solution of 2-trichloroacetyl pyrrole (1.2 kg, 5.1 mole) in acetic anhydride (6 liters) flask equipped with a mechanical stirrer was added 440 ml of fuming nitric acid over a period of 1 hour while maintaining a temperature of -40°C. The reaction was carefully allowed to warm to room temperature and stirred an additional 4 hours after which the mixture was cooled to -30°C and isopropyl alcohol (6 liters) was added. The solution was stirred at -20°C for 30 minutes during which time a white precipitate formed. The solution was allowed to

stand for 15 minutes and the resulting precipitate collected by vacuum filtration to provide (15) (0.8 kg, 54% yield). TLC (7:2 benzene/ethyl acetate) Rf 0.7; ^{1}H NMR (DMSO-d_6) δ 8.55 (d, 1 H, J = 1.7 Hz), 7.77 (d, 1 H, J = 1.7 Hz), 3.98 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 173.3, 134.7, 133.2, 121.1, 116.9, 95.0, 51.5; IR (KBr) 1694, 1516, 1423, 1314, 1183, 1113, 998, 750; FABMS *m/e* 269.936 (M + H 269.937 calc. for $C_7H_5N_2O_3Cl_3$).

Preparation of Methyl 1-methyl-4-nitropyrrole-2-carboxylate (**6**). To a solution of (**15**) (800 g, 2.9 mol) in 2.5 liters of methanol in a flask equipped with a mechanical stirrer was added dropwise a solution of NaH (60% dispersion in oil) (10 g, 0.25 mole) in 500 ml of methanol. The reaction was allowed to stir for 2 hours at room temperature, and quenched by the addition of concentrated sulfuric acid (25 ml). The reaction was then heated to reflux, until a clear light yellow solution formed. The reaction was slowly cooled to room temperature as (**6**) crystallizes as white needles, which are collected by vacuum filtration and dried *in vacuo* to provide 450 g (47% yield). TLC (ethyl acetate) Rf 0.8; ^{1}H NMR (DMSO-d_6) δ 8.22 (d, 1 H, J = 1.7 Hz), 7.22 (d, 1 H. J = 1.6 Hz), 3,88 (s, 3 H), 3.75; ^{13}C NMR (DMSO-d_6) δ 37.8, 52.2, 112.0. 123.0, 129.9, 134.6. 160.3; IR(KBr) 3148. 1718, 1541, 1425. 1317, 1226, 1195. 1116, 753; FABMS *m/e* 183.048 (M + H 184.048 calc. for $C_7H_8N_2O_4$).

Ethyl 1-methyl-4-nitroimidazole-2-carboxylate (**10**) was prepared by treatment of N-methylimidazole (**16**) with ethylchloroformate and triethylamine followed by nitration with nitric acid.

SCHEME 11



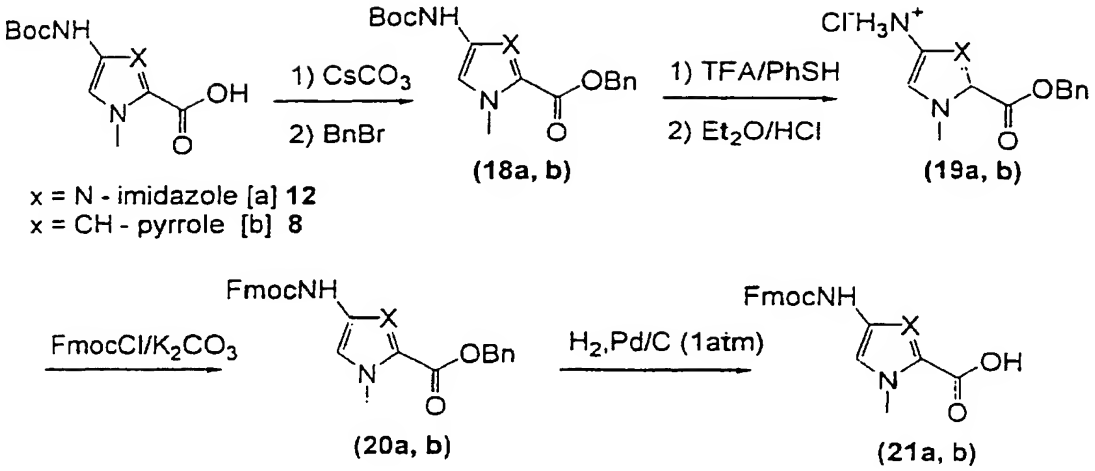
Preparation of Ethyl 1-methyl-imidazole-2-carboxylate (17). *N*-methylimidazole (16) (320 g, 3.9 mol) was combined with 2 liters of acetonitrile and 1 liter of triethylamine in a flask equipped with a mechanical stirrer and the solution was cooled to -20°C. Ethylcloroformate (1000 g, 9.2 mol) was added with stirring, keeping the temperature between -20°C and -25°C. The reaction was allowed to *slowly* warm to room temperature and stir for 36 hours. Precipitated triethylamine hydrochloride was removed by filtration and the solution was concentrated *in vacuo* at 65°C. The resulting oil was purified by distillation under reduced pressure (2 torr, 102°C) to provide (17) as a white solid (360 g, 82% yield). TLC (7:2 benzene/ethyl acetate) Rf 0.2; ^{1}H NMR (DMSO-d_6) δ 7.44 (d, 1 H, J = 2.8 Hz), 7.04 (d. 1 H, J = 2.8 Hz). 4.26 (q, 2 H, J = 3.5 Hz) 3.91 (s. 3 H), 1.26 (t, 3 H, J = 3.5 Hz); ^{13}C NMR (DMSO-d_6) δ 159.3. 129.1, 127.7, 61.0. 36.0, 14.5; IR(KBr) 3403, 3111, 2983, 1713, 1480, 1422, 1262, 1134, 1052, 922, 782, 666; FABMS *m/e* 155.083 (M + H 155.083 calc. for $C_7H_{11}N_2O_2$).

Preparation of Ethyl 4-nitro-1-methylimidazole-2-carboxylate (10). Compound (17) was carefully dissolved in 1000 ml of concentrated sulfuric acid cooled to 0°C. 90% nitric acid (1 liter) was slowly added maintaining a temperature of 0°C. The reaction was then refluxed with an

efficient condenser (-20°C) in a well ventilated hood for 50 minutes. The reaction was cooled with an ice bath, and quenched by pouring onto 10 liters of ice. The resulting blue solution was then extracted with 20 liters of dichloromethane and the combined extracts were dried and concentrated *in vacuo* to yield a tan solid which was recrystallized from 22 liters of 21:1 carbon tetrachloride/ethanol. The resulting white crystals were collected by vacuum filtration to provide pure (**10**). (103 g, 22% yield). TLC (7:2 benzene/ethyl acetate) Rf 0.5, ^{1}H NMR (DMSO-d_6) δ 8.61 (s, 1 H), 4.33 (1, 2 H, J = 6.4 Hz), 3.97 (s, 3 H), 1.29 (t, 3 H, J = 6.0 Hz); ^{13}C NMR (DMSO-d_6) δ 158.2, 145.4, 135.3, 127.4, 62.2, 37.3, 14.5; IR(KBr) 3139, 1719, 1541, 1508, 1498, 1381, 1310, 1260, 1147, 1122, 995, 860, 827, 656; FABMS *m/e* 200.066 (M + H 200.067 calc. for $C_7H_{10}N_3O_4$).

Synthesis of Fmoc-Py-OBt (**21a**) and Fmoc-Im-OBt (**21b**). The Fmoc- monomers were synthesized from the Boc- monomers as set forth in Scheme 12. Briefly, the Boc- protected monomer is converted to the cesium salt followed by treatment with benzyl bromide to yield the benzyl esters (**18**). The Boc- protecting group is then removed with trifluoroacetic acid in the presence of thiophenol and the product precipitated by the addition of HCl saturated ethyl ether. The amine hydrochloride is then treated with Fmoc-chloroformate in potassium carbonate, and the benzyl ester removed by hydrogenation to provide the Fmoc- protected monomers (**21a** and **21b**). This method is illustrated using the synthesis of the Fmoc- protected pyrrole (**21a**) as an example.

SCHEME 12



Preparation of Benzyl 4-[[tert-butyloxy)carbonyl] amino]-1-methylpyrrole-2-carboxylate (**18a, b**). To a solution of Boc-acid (8) (5 g) in 100 ml of 66% ethanol was added cesium carbonate (3.3 g in 25 ml of water). The solution was stirred for 20 minutes, filtered through glass wool, and concentrated *in vacuo* to yield the cesium salt as a solid. The solid was dissolved in 75 ml of ethanol and concentrated to dryness three times. The cesium salt was then dissolved in 500 ml of DMF and 2.6 ml of benzyl bromide was immediately added dropwise. The resulting solution was stirred at 40°C for 10 hours. After 10 hours the solution was poured into 300 ml of ice water and allowed to stand at 4°C for 1 hour. The resulting precipitate was collected by vacuum filtration to yield 6.59 (93%) of the Boc protected benzyl ester (**18a**). ^{1}H NMR (DMSO-d_6) δ 9.1 (s, 1 H), 7.4 (m, 5 H), 7.1 (d, 1 H), 6.8 (d, 1 H), 5.2 (s, 2 H), 3.8 (s, 3 H), 1.4 (2, 9 H).

Preparation of Benzyl 4-amino-1-methylpyrrole-2-carboxylate (**19a**). To a solution of the Boc-benzyl ester(**18a**) (5 g) in 20 ml of

dichloromethane was added 20 ml of 65% TFA/CH_2Cl_2/0.5 M PhSH. The reaction was allowed to stir for 1 hour, and then partitioned between 100 ml of 1M LiOH and 100 ml of ethyl ether. The layers were separated and the aqueous layer was extracted with ethyl ether (5 x 20 ml). HCl (g) was bubbled through the combined organics and the product collected by vacuum filtration to yield 3.2 g (76%) of benzylamine (**19a**). ^{1}H NMR (DMSO-d_6) δ 10.1 (br s, 3 H), 7.4 (m, 5H), 7.2 (d, 1 H), 6.8 (d, 1 H), 5.2 (s, 2 H), 3.8 (s, 3 H).

Preparation of 4-[[(9-fluorenylmethyl)carbonyl]amino]-1-methylpyrrole-2-carboxylate (**20a**). To a solution of the benzylamine (**19a**) (1 gram) in dichloromethane cooled at 0°C was added DIEA (1.4 ml) and 9-fluorenylmethylchloroformate (973 mg). The reaction was allowed to stir for 30 minutes. The reaction was worked up using standard methods to yield 1.8 g (88%) of benzylester (**21a**). ^{1}H NMR (DMSO-d_6) δ 9.5 (s, 1 H), 7.9 (d, 2 H), 7.7 (d, 2 H), 7.3 (m, 9 H), 7.1 (s, 1 H), 6.7 (s, 1 H), 5.2 (s, 2 H), 4.4 (d, 2 H), 4.2 (t, 1 H), 3.8 (s, 3 H).

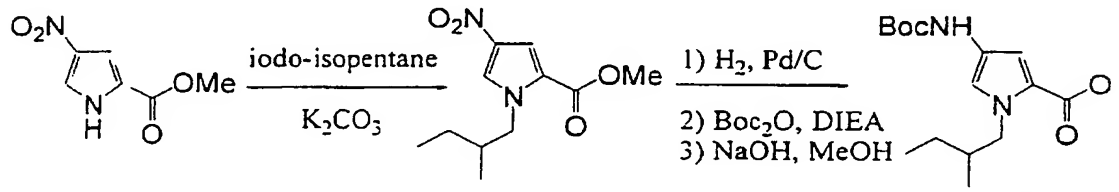
Preparation of 4-[[(9-fluorenylmethyl)carbonyl]amino]-1-methylpyrrole-2-carboxylic acid (**21a**). To a solution of the benzylester (**20a**) (900 mg) dissolved in THF (10 ml) was added 10% Pd/C (100 mg). The solution was hydrogenated (1 atm) for 19 hours and worked up using standard methods to yield 580 mg (80%) of compound (**21a**). ^{1}H NMR (DMSO-d_6) δ 9.4 (s, 1 H), 8.0 (m, 2 H), 7.8 (m, 2 H), 7.3 (m, 4 H), 7.1 (s, 1 H), 6.8 (s, 1 H), 4.6 (m, 2 H), 4.3 (m, 1 H), 3.8 (s, 3 H).

Preparation of N-substituted monomers.

Preparation of *N*-2-methyl-butyl-4-[[(*tert*-butyloxy)carbonyl]amino]-2-carboxylic acid (**24**). *N*-2-methyl-propyl-4-[[(*tert*-butyloxy)carbonyl]amino]-2-carboxylic acid **24** was synthesized as

outlined in Scheme 13. Briefly, methyl 4-nitropyrrole-2-carboxylate **22**, prepared as described below, was alkylated by refluxing with the appropriate alkyl halide in acetone in the presence of potassium carbonate. The ester was then hydrogenated and hydrolyzed to provide the modified monomer **24** which is ready for use in solid phase synthesis.

SCHEME 13



(22) (23) (24)

Preparation of Methyl *N*-2-methyl-butyl-4-nitropyrrole-2-carboxylate (**23**). Methyl 3-nitropyrrole-2-carboxylate (**22**) (2.7 g, 15.9 mmol), potassium carbonate (6.5 g), and iodo-2-methylbutane (5.2 ml) were dissolved in 100 ml of acetone and refluxed for 10 hours. The reaction mixture was then cooled to room temperature, concentrated *in vacuo*, partitioned between 200 ml of dichloromethane and 200 ml of water and extracted with dichloromethane (2 x 200 ml). The combined organic layers were dried (sodium sulfate) and concentrated *in vacuo*. The resulting yellow oil was purified by flash chromatography to provide the substituted nitro pyrrole **23** (2.5 g, 70% yield). ^{1}H NMR (DMSO-d_6) δ 8.30 (d, 1 H, J = 2.0 Hz), 7.33 (d, 1 H, J = 1.9 Hz), 4.15 (d, 2 H, J = 7.4 Hz), 3.77 (s, 3 H), 2.03 (m, 1 H, J = 3.1 Hz), 0.80 (d, 6 H, J = 6.7 Hz); ^{13}C NMR (DMSO-d_6) δ 160.30, 134.79, 129.80, 122.40, 112.76, 105.00, 56.63, 52.41, 29.72, 19.73; FABMS, 226.096 calcd, 226.095 found.

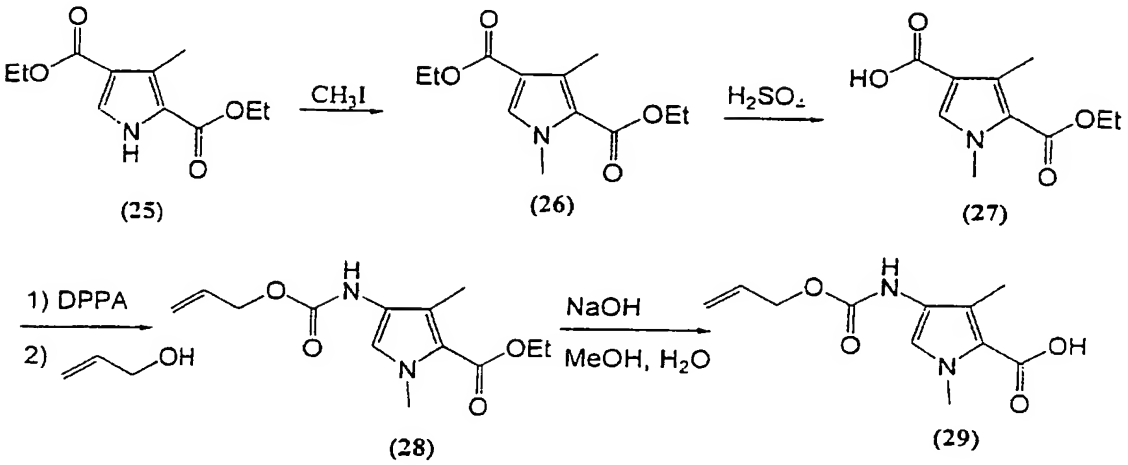
Preparation of *N*-2-methylbutyl-4-[[(*tert*-butyloxy)carbonyl]amino]-2-carboxylic acid (**24**). A solution of methyl *N*-2-methylbutyl-4-nitropyrrole-2-carboxylate **23** (2.3 g, 0.98 mmol) in 20 ml of DMF was treated with a Pd/C catalyst (10%, 500 mg) and the mixture was hydrogenated in a Parr bom apparatus (500 psi H_2) for 7 hours. After 7 hours, Boc-anhydride (2.95 g, 13.5 mmol) was added followed by DIEA (5 ml) and the reaction was stirred overnight. The reaction mixture was then partitioned between 200 ml of water and 200 ml of ethyl ether and extracted with ethyl ether (2 x 200 ml). The combined organics were dried over sodium sulfate and concentrated *in vacuo* to yield a yellow oil. The resulting yellow oil was dissolved in 30 ml of methanol and 30 ml of 1M NaOH was added. The solution was heated at 50°C for 6 hours, cooled to room temperature, extracted with ethyl ether (2 x 200 ml), acidified to pH 0 with sodium bisulfate, and extracted with ethyl ether (3 x 200 ml). The combined acidic extracts were dried (sodium sulfate) and concentrated *in vacuo* to yield a white solid. (1.2 g, 41% yield). ^{1}H NMR (DMSO-d_6) δ 12.1 (br s, 1 H), 9.09 (s, 1 H), 7.05 (s, 1 H), 6.59 (s, 1 H), 4.10 (d, 2H, J = 7.1 Hz), 3.35 (s, 2 H), 1.92 (m, 1 H), 1.44 (s, 9 H), 1.70 (d, 6 H. J = 6.4 Hz); ^{13}C NMR (DMSO-d_6) 175.0, 161.9, 112.9, 119.4, 118.5, 108.3, 108.2, 55.0, 30.1, 28.4, 19.7.

Preparation of Monomers Substituted at the 3-Position of the Pyrrole.

Preparation of 3-methyl substituted pyrroles. Scheme 14 outlines a general synthesis of a 3-methyl substituted pyrrole. In this example the amine is protected with an allyloxycarbonyl group. Briefly, diethylester (**25**) is methylated and hydrolyzed to yield monoacid **27**. Monoacid **27** is reacted with

allyl alcohol/DPPA to yield allyl ester (**28**) which is hydrolyzed to form compound **29**.

SCHEME 14



2, 4-Dicarbethoxy-1, 3-dimethylpyrrole (**26**). A solution of 2, 4-dicarbethoxy-3 methylpyrrole (**25**) (2.44 g, 10.9 mmol), K_2CO_3 (9.9 g), and iodomethane (55 ml) in 750 ml acetone was refluxed at 50°C for 15 hours. The solution was concentrated *in vacuo*, partitioned between 450 ml of dichloromethane and 600 ml of water. The aqueous layer was extracted with CH_2Cl_2 (2 x 500 ml), dried (sodium sulfate), and concentrated *in vacuo* to yield a brown oil that solidified upon standing at room temperature for several minutes and was used without further purification. (2.37 g. 91% yield). TLC (benzene) Rf 0.3; ^1H NMR (CD_2Cl_2) δ 7.31 (s, 1H), 4,23 (m. 4H), 3,84 (s, 3H). 2.54 (2, 3H), 1.33 (m, 6H).

1, 3-Dimethyl-4-carboxyl-2-carbethoxypyrrole (**27**). 2, 4-Dicarbethoxy-1, 3-dimethyl pyrrole (**26**) (9.24 g, 38.6 mmol) was suspended in

120 ml of concentrated sulfuric acid and vigorously stirred for 32 minutes at room temperature. The mixture was then precipitated by pouring into 2 liters of ice. The product was collected by vacuum filtration, washed with water (5 x 250 ml), and dried *in vacuo* to provide a white sand. (7.38 g, 91% yield). ^1H NMR ($CDCl_3$) δ 7.42 (s, 1H), 4.35 (m, 2H), 3.89 (s, 3H), 2.59 (s, 3H), 1.39 (t, 3H, J=7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 237.1, 188.9, 184.4, 163.4, 161.6, 160.8, 159.9, 105.0, 46.6, 15.6.

Preparation of Ethyl 4-[(Allyloxycarbonyl)amino]-1,3-dimethylpyrrole-2-carboxylate (**28**). 1,3-dimethyl-4-carboxyl-2-carbethoxypyrrole (**27**) (2.19 g, 10.4 mmol), triethylamine (1.45 ml, 10.4 mmol) and diphenylphosphorylazide (DPPA) (Rappnport) (2.234 ml, 10.4 mmol) were dissolved in 31 ml of DNA synthesis grade CH_3CN (Fisher). The solution was refluxed for 4.5 hours under argon, after which allyl alcohol (31 ml) was added. The solution was refluxed for an additional 22 hours under argon. After 22 hours, the solution was concentrated *in vacuo*, partitioned between 250 ml water and 250 ml diethyl ether, washed several times with 10% Na_2CO_3, 1M HCl, and water. The organic layer separated, dried (sodium sulfate), and concentrated to provide yellow crystals **28** (1.59 g, 58% yield). TLC (ethyl acetate) Rf 0.9; ^1H NMR (DMSO) δ 8.77 (br, 1H), 7.05 (s, 1H) 5.95 (m, 1H), 5.36 (d, 1H, J=17.1 Hz), 5.22 (d, 1H, J=10.4 Hz), 4.54 (d, 2H, J=5.04 Hz), 4.19 (1, 2H, J=7.1 Hz), 3,75 (s, 3H), 2.102 (s, 3H), 1.27 (t, 3H, J=7.1 Hz); ^{13}C NMR (DMSO) δ 161.3, 154.6, 133.8, 123.0, 121.1, 117.4, 64.8, 59.4, 37.1, 14.5, 10.5.

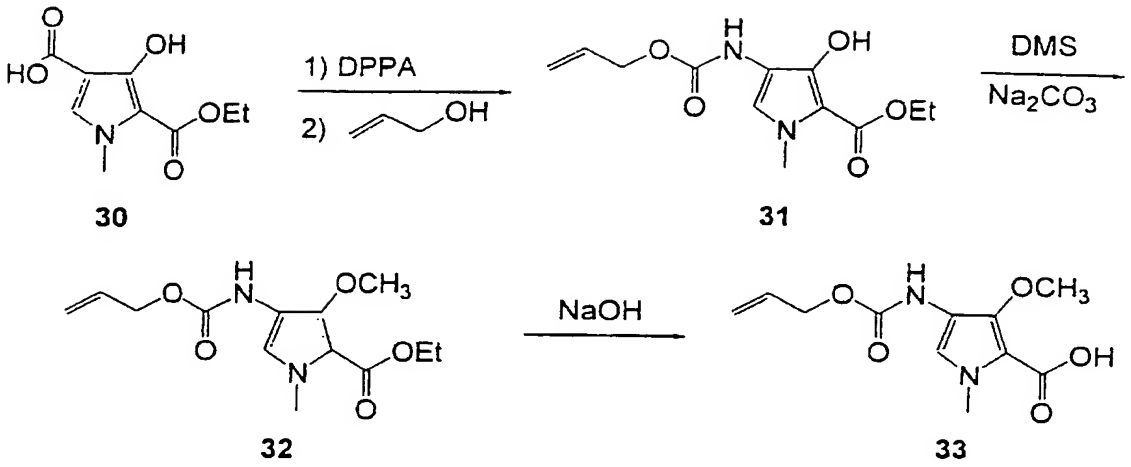
Preparation of 4-[(Allyloxycarbonyl)amino]-2-carboxyl-1,3-dimethylpyrrole (**29**). Ethyl 4-[(allyloxycarbonyl)amino]-1,3-dimethylpyrrole-2-carboxylate (**28**) (1.00 g, 3.75 mmol) was suspended in 6 ml water.

Methanol was added with vigorous stirring until all starting material dissolved. 8 M NaOH (8 ml) was added, and the solution was stirred for five hours at 50°C. The reaction mixture was allowed to cool to room temperature, and 1M HCl added to approximately pH 2 while solution cooled in ice bath to precipitate out product. The product was collected by vacuum filtration, washed once with water, and dried *in vacuo* to yield 0.800 g (90% yield) of compound **29**. TLC (ethyl acetate) Rf 0.8; ^{1}H NMR (DMSO) δ 12.23 (br, 1H), 8.73 (s, 1H), 7.01 (s, 1H), 5.93 (m, 1H), 5.33 (d, 1H, J=17.1 Hz), 5,19 (d, 1H, J=10.5 Hz), 4.53 (d, 2H, J=5.5 Hz) 3.74 (s, 3H), 2.09 (s, 3H); mass. spec. Calc. 238.0954 Found 238.0952.

Scheme 15 illustrates two syntheses of 3-hydroxy substituted pyrrole monomers. Both syntheses utilize the previously described ethyl N-methyl-2,4-carboxy-3-hydroxypyrrole (**30**) as a starting material (Momose *et al.* (1978) Chem. Pharm. Bull. 26:2224). In the first approach, the acid is converted to the allyl carbamate (**31**) using DPPA and allyl alcohol in a modified Curtius reaction. The hydroxy group is then protected as a methyl ester with DMS, and the ethyl ester subsequently hydrolyzed with sodium hydroxide to yield compound (**33**).

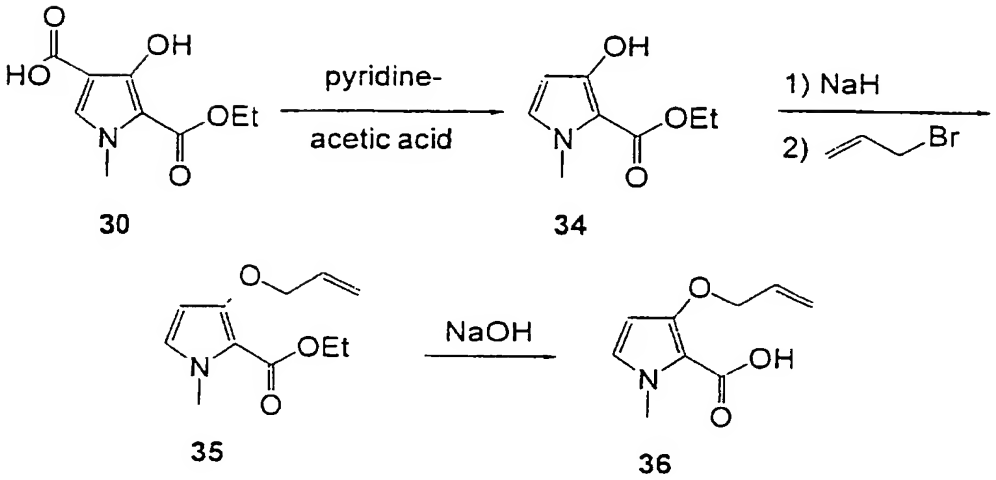
The second approach produces a 3-substituted monomer which is appropriate as an N-terminal capping reagent. In this approach, ethyl N-methyl-2,4-carboxy-3-hydroxypyrrole (**30**) is first decarboxylated under acidic conditions. The hydroxy group is then protected as the allyl ether, and the ethyl ester subsequently hydrolyzed to yield compound (**36**).

SCHEME 15



30 31 32 33

SCHEME 16



30 34 35 36

Preparation of Ethyl 4-allyloxycarbonyl-3-hydroxy pyrrole-2-carboxylate (31). Ethyl N-methyl-2,4-carboxy-3-hydroxypyrrole (**30**) (500 mg, 2.36 mmol) was dissolved in 7 ml of acetonitrile. Triethylamine (329 µl) was added to this solution followed by DPPA (508 µl). The mixture was refluxed for 1.5 hours. after which allyl alcohol was added (7.1 ml) and the mixture was refluxed for an additional 17 hours. The reaction mixture was worked up using standard methods and the product was purified by flash chromatography (2% MeOH/$CHCl_3$/AcOH) to yield 250 mg, (39%) of compound (**31**). FABMS (low res. 268 found, 268 calc.)

Preparation of 4-allyloxycarbonyl-3-methoxy pyrrole-2-carboxylic acid (33). Compound **32** was prepared from Compound **31**, using standard means (Dms, Na_2CO_3) (Greene (1991) in Protecting Groups in Organic Synthesis, John Wiley & Sons 2nd Ed., NY, NY) To a solution of ethyl 4-allyloxycarbonyl-3-methoxy pyrrole-2-carboxylate (**32**) (190 mg. 675 µmol) in 12 ml of ethanol was added 0.1 sodium hydroxide (6.8 ml). The solution was refluxed for 3 days and worked up using standard methods.

Preparation of Ethyl 3-hydroxy pyrrole-2-carboxylate (34). To a solution of ethyl N-methyl-2,4-carboxy-3-hydroxypyrrole (**30**) (1000 mg. 4.7 mmol) in pyridine (8 ml) was added acetic acid (8 ml) and the solution was refluxed for 6 hours. The reaction mixture was concentrated onto silica gel and purified by flash chromatography (25%) to yield 580 mg (73%) of compound (**34**).

Preparation of Ethyl 3-allyloxy pyrrole-2-carboxylate (35). To a solution of ethyl 3-hydroxypyrrole-2-carboxylate (**34**) (580 mg) in benzene (12 ml) was added sodium hydride (387 mg). The suspension was heated at 60-70°C for 1 hour. A solution of allyl bromide in benzene was then added and

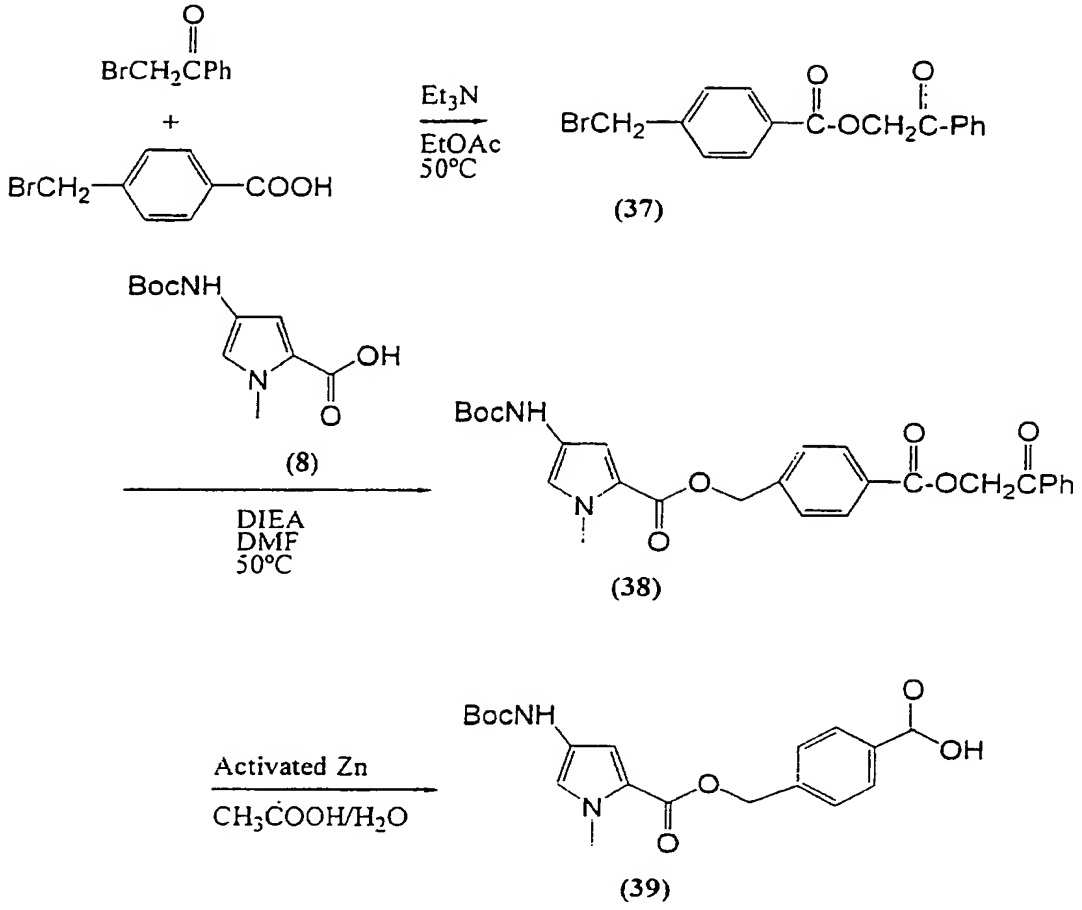
the mixture heated at 70-80°C for ___??___ hour. The reaction was concentrated onto silica gel and purified by flash chromatography (25% EtOAc/hexane) to yield 300 mg (50%) of compound (**35**).

Example 2. Activation of Amino Acids. To activate the various amino acids 1.0 mmol of the appropriate amino acid was dissolved in 2 ml DMF. HOBt (135 mg, 1.0 mmol) was added followed by DCC (263 mg, 1 mmol) and the solution lightly shaken for at least 30 minutes. The precipitated DCU by product was filtered before addition to the coupling reaction.

Example 3. Preparation of Boc-Pyrrole-PAM and Boc Pyrrole-BAM-Resins (24) and (25)

Preparation of Resin Linkage Agents **39** and **40**. Resin linkage agents **39** and **40** were prepared in three steps according to the published procedures of Merrifield, using Boc-Py-COOH as the amino acid (Mitchell *et al.* (1978) J. Org. Chem. 43:2845-2852) as outlined in Scheme 16.

SCHEME 16



Preparation of 4-(Bromomethyl)benzoic acid phenacyl ester (37). Triethylamine (16 ml, 115 mmol) and bromoacetophenone (22.9 g, 115 mmol) were dissolved in 450 ml of ethyl acetate. The solution was stirred at 50°C and 4-(bromomethyl)benzoic acid (17.5 g, 155 mmol) was added in seven equal portions over a three hour period. Stirring was continued for an additional 8 hours at 50°C. Precipitated triethylaminehydrobromide was removed by filtration, and the ethyl acetate solution was washed with (3 x 150 ml each) of 10% citric acid, brine, saturated sodium bicarbonate and brine. The organic

phase was dried with sodium sulfate and concentrated *in vacuo*. The residue was recrystallized from dichloromethane-petroleum ether to give fine white crystals (10.2 g, 27% yield). ^{1}H NMR (DMSO-d_6) δ 7.99 (m, 4H), 7.69-7.54 (m, 5H), 5.74 (s, 2H), 4.77 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 193.7, 165.9, 144.6, 134.9, 131.2, 131.0, 130.7, 130.6, 130.2, 129.9, 128.8, 128.6, 68.2, 34.0.

Preparation of Boc-pyrrolyl-4-(oxymethyl)benzoic acid phenacyl ester (38). A solution of Boc-pyrrole-OH (**8**)(2.9 g 12 mmol), 4-(bromomethyl)benzoic acid phenacyl ester (**22**) (4 g, 12 mmol) and diisopropylethylamine (3.0 ml, 16.8 mmol) in 60 ml of DMF were stirred at 50°C for 6 hours. The solution was cooled and partitioned between 400 ml of water and 400 ml of ethyl ether. The ether layer was washed with (2 x 200 ml each) of 10% citric acid, brine, saturated sodium bicarbonate and brine. The organic phase was dried with sodium sulfate and concentrated *in vacuo* to yield compound **38** as light white foam which was used without further purification (5.4 g, 97% yield). TLC (2:3 hexane/ethyl acetate) Rf 0.6; ^{1}H NMR (DMSO-d_6) δ 9.14 (s, 1H), 8.03 (m, 4H), 7.67 (m, 1H), 7.55 (m, 4H), 7.13 (s, 1H), 6.72 (d, 1H, J = 1.5 Hz), 5.74 (s, 1H), 5.32 (s, 1H), 3.79 (s, 3H), 1.42 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 193.2, 165.5, 160.4, 153.2, 143.1, 134.5, 130.1, 129.5, 128.3, 128.2, 123.8, 120.3, 118.8, 108.2, 79.1, 67.7, 64.6, 36.7, 28.6.

Preparation of Boc-pyrrolyl-4-(oxymethyl)phenylacetic acid phenacyl ester. This compound was prepared by the method described above for Boc-pyrrolyl-4-(oxymethyl)benzoic acid phenacyl ester. The product was purified by crystallization with hexane:ethyl acetate (3:1) as long needles (6.1 g, 44.5%). TLC (3:1 hexane/ethyl acetate) Rf 0.2; ^{1}H NMR (DMSO-d_6) δ 9.11 (s, 1H), 7.93 (d, 2H, J = 8.2), 7.67 (t, 1H, J = 7.0), 7.52 (t, 2H, J = 7.9), 7.35 (m, 4H), 7.10 (s, 1H), 6.67 (s, 1H), 5.50 (s, 2H), 5.22 (s, 2H), 5.19 (s, 2H), 3.83

(s, 3H), 1.42 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 193.1, 171.2, 160.6, 153.2, 135.7, 134.4, 130.1, 129.4, 128.5, 128.3, 123.7, 120.0, 119.0, 108.0, 79.0, 67.4, 65.1, 36.7, 28.6.

Preparation of Boc-pyrrolyl-4-(oxymethyl)benzoic acid (39). Boc-pyrrolyl-4-(oxymethyl)benzoic acid phenacyl ester (**38**) (3 g, 5.9 mmol) was dissolved in 90 ml of acetic acid and water (80:20). Activated zinc dust (9.6 g, 147 mmol) was added and the reaction was stirred for 18 hours at room temperature. The zinc was removed by filtration and the reaction mixture was partitioned between 200 ml of ethyl ether and 200 ml of water. The layers were separated and the aqueous layer was extracted with another 200 ml ethyl ether. The ether layers were combined and washed with (5 x 100 ml) of water. The combined organics were dried with sodium sulfate, concentrated *in vacuo*, and azeotroped with (6 x 100 ml) of benzene. The product was purified by flash chromatography with a gradient of 2:1 hexane:ethyl acetate to ethyl acetate to give a yellow oil (1.9 g, 54%) of compound **39**. TLC (ethyl acetate) Rf 0.7.

Preparation of Boc-pyrrolyl-4-(oxymethyl) phenylacetic acid (40). Prepared in a manner analogous to **39**, yielding **40** as a yellow oil in 78% yield.

Preparation of Boc-aminoacyl-pyrrolyl-4-(oxymethyl)-BAM-resin (41). BAM linker acid (**39**) (1 g, 2.6 mmol) was dissolved in 6.5 ml of DMF/HOBt (382 mg, 2.8 mmol). DCC (735 mg, 2.8 mmol) was added and the reaction mixture was shaken at room temperature. After 4 hours the precipitated DCU byproduct was filtered and the reaction mixture was added to 3 grams aminomethyl-polystyrene-resin (0.7 mmol/gram substitution) previously swollen for 30 minutes in DMF. Diisopropylethylamine (913 µl,

5.3 mmol) was added and the reaction was shaken for 12 hours. After 12 hours the resin was determined by the ninhydrin test to be approximately 0.3 mmol/gram substituted. At this time the resin was washed with DMF and the remaining amine groups were capped by acetylation (2x) with excess acetic anhydride capping solution. The resin was washed with DMF, dichloromethane and MeOH and dried *in vacuo*.

Preparation of Boc-aminoacyl-pyrrolyl-4-(oxymethyl)-PAM-resin (42). Boc-Py-PAM-resin (**42**) (0.3 mmol/g substitution) was prepared using PAM linker acid **40** as described above for the BAM resin.

Example 4. Solid Phase Polyamide Synthesis

Preparation of Boc-PyPy-G-PyPyPy-G-PAM-resin. Boc-G-PAM-resin (1.25 g, 0.25 mmol amine) was shaken in DMF for 15 minutes and drained. The N-boc group was removed by washing with dichloromethane for 1 minute, followed by washing with 65% TFA/CH_2Cl_2/0.5M PhSH for 30 seconds, shaking in 65% TFA/CH_2Cl_2/0.5 PhSH for 60 seconds, washing with 65% TFA/CH_2Cl_2/PhSH for 30 seconds, and shaking in 65% TFA/CH_2Cl_2/PhSH for 20 minutes. The trifluoroacetic acid deprotection mixture (65% TFA/CH_2Cl_2/0.5M PhSH) was prepared by combining and shaking a mixture of trifluoroacetic acid (TFA) (290 ml), dichloromethane (150 ml), and thiophenol (23 ml, 225 mmol). The resin was washed for 1 minute with dichloromethane, 30 seconds with DMF, and shaken for 1 minute in DMF. The resin was then drained completely and activated acid, Boc-Py-OBt, (1 mmol, 4 eq., prepared as described in Example 2) in 2 ml DMF was added followed by DIEA (355 µl, 8 eq.) and the resin *shaken vigorously* to make a slurry. After shaking the reaction was allowed to proceed for 45 minutes after

which the reaction vessel was washed with DMF for 30 seconds completing a single reaction cycle. Five additional cycles were performed adding, Boc-Py-OBt, Boc-Py-OBt, Boc-G-OBt, Boc-Py-OBt and Boc-Py-OBt to give Boc-PyPy-G-PyPyPy-G-PAM-Resin. The resin was washed with DMF (1 minute), dichloromethane (1 minute) and methanol (1 minute) and dried *in vacuo*. This compound was then used to synthesize ImPyPy-G-PyPyPy-G-Ed (**2a**). ImPyPy-G-PyPyPy-G-Dp (**2b**), AcImPyPy-G-PyPyPy-G-Dp (**2c**), ImPyPy-G-PyPyPy-G-Ta-EDTA, ImPyPy-G-PyPyPy-G-Ta, and AcImPyPy-G-PyPyPy-G-Ta (**2d**) as described below.

Preparation of AcImPyPy-G-PyPyPy-G-Dp (**2c**). A sample of Boc-PyPy-G-PyPyPy-G-PAM-resin (600 mg, about 100 μmole) was placed in a reaction vessel and shaken in DMF for 20 minutes. The resin was subsequently drained and subjected to an additional coupling cycle with Boc-Im-OBt, as described above, to add an N-terminal Boc imidazole. The N-Boc group was removed as described above and the resin was washed with dichloromethane (30 seconds) and DMF (1 minute). The resin was then treated with 4 ml of an acetylation mixture (acetylation mixture: DMF (4 ml), DIEA (710 μl, 4.0 mmol), and acetic anhydride (380 μl, 4.0 mmol) combined immediately before use) for 1 hour. The reaction vessel was then washed with DMF (1 minute), dichloromethane (1 minute) and methanol (1 minute) and dried *in vacuo* to yield AcImPyPy-G-PyPyPy-G-PAM-Resin. The resin (180 mg, 29 μmol) was weighed into a glass scintillation vial and treated with 1.5 ml of DMF, after 10 minutes, 1.5 ml of dimethylaminopropylamine (Dp) was added and the mixture was shaken at 37°C for 12 hours. The resin was removed by filtration through an ISOLAB polypropylene filter and washed with 11 ml of water. The DMF solution and the water washes were combined.

Seven milliliters of the combined solution was loaded on a C_{18} preparatory HPLC column, the column was washed for 2 minutes in 0.1% TFA at 8 ml/min. to remove the DMF, followed by addition of a second 7 ml portion, which was also washed free of DMF with 0.1% TFA for 2 minutes at 8 ml/min. No flushing of the polyamide occurs so long as the injection solution is less than 20% v/v DMF. The polyamide was then eluted in 100 minutes with a gradient of 0.25% CH_3CN per minute. The polyamide was collected in 4-5 separate 8 ml fractions, the purity of the individual fractions was verified by HPLC and ^1H NMR, to give AcImPyPy-G-PyPyPy-G-Dp (**2c**) (11.8 mg, 39%).

Characterization of **2c**. HPLC, r.t. 26.9; UV(H_2O/DMSO)$\lambda_{max}(\epsilon)$, 246 (45,200), 304 (50,200) (Extinction coefficients (ϵ) were determined by taking a 5 µl aliquot from two separate NMR samples, diluting to 1 ml with water and measuring the UV spectrum. The presence of 0.5% DMSO does not result in a significant change in the measured extinction coefficient.); ^1H NMR (DMSO-d_6) δ 10.24 (s, 1H), 9.98 (s, 1H), 9.96 (s, 1H), 9.94 (s, 1H), 9.92 (s, 1H), 9.90 (s, 1H), 9.2 (br s, 1H, $CF_3COO\mathbf{H}$), 8.29 (m, 2H, G-NH and G-NH), 8.02 (t, 1H, J = 6.6 Hz, PyCONH-G), 7.41 (s, 1H), 7.26, (d, 1H, J = 1.7 Hz), 7.23 (m, 3H), 7.16 (d, 1H, J = 1.8 Hz), 7.14 (d, 1H, J = 1.7 Hz), 7.05 (d, 1H J = 1.8 Hz), 6.94 (m, 3H), 3.93 (s, 3H), 3.89 (d, 2H, J = 3.9 Hz, Gly CH_2), 3.84 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.72 (d, 2H, J = 4.9 Hz, GlyCH_2), 3.14 (q, 2H, J = 5.0 Hz), 3.03 (q, 2H, J = 6.0 Hz), 2.74 (d, 6H, J = 6.0 Hz, $CH_2N(CH_3)_2$), 2.00 (s, 3H, CH_3CONH), 1.77 (quintet, 2H, J = 4.6 Hz, $CH_2CH_2N(CH_3)_2$); MALDI-TOF MS, calcd M^+H 993.1. found 993.8.

Preparation of ImPyPy-G-PyPyPy-G-Dp (**2b**). A sample of Boc-PyPy-G-PyPyPy-G-PAM-Resin (600 mg, about 100 µmole) was placed in a reaction vessel and shaken in DMF for 15 minutes. The N-Boc group was

removed with TFA as described above and the resin was washed with dichloromethane (30 seconds) and DMF (1 minute) and was treated with the HOBt ester of *N*-methyl imidazole-2-carboxylic acid (about 16 equivalents, prepared as described below) and DIEA (155 µl, 16 eq.) for 2 hours. The resin was washed with DMF, dichloromethane and MeOH (1 minute each) and dried *in vacuo* to yield ImPyPy-G-PyPyPy-G-PAM-Resin. It should be noted that polyamides capped with *N*-methyl imidazole-2-carboxylic acid tend to give false positives for the picric acid test even when reactions are >99% complete as determined by stepwise HPLC analysis. The crude product was cleaved from the resin (180 mg, 29 µmole) with dimethylaminopropylamine and purified as described for (**2c**) to yield ImPyPy-G-PyPyPy-G-Dp (**2b**) (12 mg, 40% recovery).

Characterization of **2b**. HPLC, r.t. 26.9; UV(H_2O/DMSO)$\lambda_{max}(\epsilon)$, 246 (41,100), 304 (48,400); ^{1}H NMR (DMSO-d_6) δ 10.49 (s, 1H), 9.98 (s, 1H), 9.95 (s, 1H), 9.92 (s, 1H), 9.89 (s, 1H), 9.2 (br s, 1H, CF_3COOH), 8.30 (m, 2H, Gly-NH and Gly-NH), 8.06 (t, 1H, J = 5.8 Hz, PyCONH-Gly), 7.40 (s, 1H), 7.24, (d, 1H, J = 1.7 Hz), 7.23 (m, 3H), 7.17 (m, 2H), 7.06 (m, 2H), 6.94 (m, 3H), 3.99 (s, 3H), 3.89 (d, 2H, Gly CH_2), 3.84 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.72 (d, 2H, J = 4.3 Hz, GlyCH_2), 3.13 (q, 2H, J = 5.7 Hz), 3.01 (q, 2H, J = 5.2 Hz), 2.76 (d, 6H, J = 4.3 Hz, $CH_2N(CH_3)_2$), 1.77 (quintet, 2H, J = 7.4 Hz, $CH_2CH_2N(CH_3)_2$); MALDI-TOF MS, calc. for (M^-H^+) 936.0, found 935.7.

Preparation of ImPyPy-G-PyPyPy-G-Ed (**2a**). ImPyPy-G-PyPyPy-G-PAM-Resin (180 mg, 29 µmole, synthesized as described for **2b** was shaken in 1.5 ml of DMF. After 20 minutes, ethylenediamine (Ed) (1.5 ml) was added and the mixture was shaken at 37°C for 12 hours. The crude

product was purified as described for **2c** to yield ImPyPy-G-PyPyPy-G-Ed (**2a**) (9 mg, 39%).

Characterization of **2a**. HPLC, r.t. 24.2; UV(H_2O/DMSO)$\lambda_{max}(\epsilon)$, 246 (44,400). 304 (51,300); ^{1}H NMR (DMSO-d_6) δ 10.49 (s, 1H), 9.97 (s, 1H), 9.94 (s, 1H), 9.92 (s, 1H), 9.88 (s, 1H), 8.30 (t, 1H, J = 2.6 Hz), 8.23 (t, 1H, J = 5.6 Hz), 8.04 (t, 1H, J = 3.1 Hz), 7.73 (br s, 1H), 7.40 (s, 1H), 7.28 (d, 1H, J = 1.7 Hz), 7.23 (d, 1H, J = 1.7 Hz), 7.22 (m, 2H), 7.15 (m, 2H), 7.05 (m, 2H), 6.95 (m, 3H). 3.98 (s, 3H), 3.88 (d, 2, J = 4.1), 3.83 (s, 3H), 3.82 (m, 6H), 3.80 (s, 3H), 3.79. (s, 3H), 3.76 (m, 2H), 3.29 (m, 2H), 2.84 (m, 2H); MALDI-TOF MS, calc. for (M^+H^+) 893.9, found 894.9.

Preparation of AcImPyPy-G-PyPyPy-G-Ta (**2d**). AcImPyPy-G-PyPyPy-G-PAM-Resin (180 mg, 29 μmole, synthesized as described for **2c**) was treated with 1.5 ml of DMF. After 20 minutes, 1.5 ml of 3,3'-diamino-N-methylpropylamine (Ta) was added and the mixture was shaken at 37°C for 12 hours and purified as described for (**2c**) to yield AcImPyPy-G-PyPyPy-G-Ta (**2d**) (6.7 mg, 23% yield).

Characterization of **2d**. HPLC, r.t. 25.9: UV(H_2O/DMSO)$\lambda_{max}(\epsilon)$, 246 (43,600), 304 (51,800): ^{1}H NMR (DMSO-d_6) δ 10.23 (s, 1H). 9.99 (s, 1H), 9.96 (s, 1H), 9.94 (s, 1H). 9.91 (s, 1H), 9.89 (s. 1H) 9.53 (br s, 1H. CF_3COOH). 8.28 (m, 2H, J = 6.1 Hz. Gly-NH and Gly-NH). 8.04 (t, 1H, J = 5.3 Hz, PyCONH-Gly), 7.79-7.82 (br s, 3H, CH_2NH_3), 7.41. (s, 1H), 7.25 (d. 1H, J=1.7 Hz). 7.23 (d, 1H, J = 1.7 Hz). 7.22 (d, 1H, J = 1.7 Hz), 7.20 (d, 2H. J = 1.7 Hz), 7.15 (d, 1H, J = 1.7 Hz), 7.13 (d, 1H, J = 1.7 Hz). 7.05 (d, 1H, J = 1.7 Hz), 6.94 (m. 2H). 6.92 (d, 1H, J = 1.7 Hz), 3.89 (s, 3H), 3.88 (d, 2H, $GlyCH_2$), 3.85 (s, 3H), 3.84 (s, 3H), 3.82 (s. 3H), 3.80 (s. 3H), 3.79 (s, 3H), 3.71 (d. 2H. J = 5.5 Hz. $GlyCH_2$), 3.37 (m. 2H). 3.13 (m, 4H), 2.80 (m.

2H), 2.73 (d, 3H, J = 3.3 Hz, NCH_3), 2.01 (s, 3H, CH_3CO), 1.89 (m, 2H), 1.77 (m, 2H); MALDI-TOF MS, calc. for (M^+H^+) 1036.2, found 1036.2.

AcImPyPy-G-PyPyPy-G-Ta-EDTA (**2e**). To a solution of AcImPyPy-G-PyPyPy-G-PAM-resin (synthesized as described for **2c**) (3.0 mg, 2.5 μmole) in 750 μl of DMSO was added 750 μl NMP, followed by EDTA monoanhydride (30 mg, 118 μmole) and the solution was heated at 37°C. After 2 hours, 13 ml of water was added and the reaction was purified by preparatory HPLC as described above. The EDTA derivative eluted at 120 minutes to give AcImPyPy-G-PyPyPy-G-Ta-EDTA (**2e**) (1.1 mg, 32% yield).

Characterization of **2e**. HPLC, r.t. 27.8; UV(H_2O/DMSO)λ_{max}, 246, 304; ^{1}H NMR (DMSO-d_6) δ 10.23 (s, 1H), 9.99 (s, 1H), 9.96 (s, 1H), 9.94 (s, 1H), 9.91 (s, 1H), 9.89 (s, 1H) 9.25 (br s), 8.43 (t, 1H), 8.33 (m, 2H), 8.06 (t, 1H) 7.41, (s, 1H), 7.26 (d, 1H, J = 1.7 Hz), 7.22 (d, 1H, J = 1.7 Hz), 7.21 (d, 1H, J = 1.7 Hz), 7.20 (d, 2H, J =1.7 Hz), 7.15 (d, 1H, J = 1.7 Hz), 7.13 (d, 1H, J = 1.7 Hz), 7.07 (d, 1H, J = 1.7 Hz), 6.94 (m, 2H), 6.92 (d, 1H, J = 1.7), 3.93 (s, 3H), 3.88 (d, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.71 (d, 2H), 3.65 (m 4H), 3.26 (m, 10H), 3.13 (m, 4H), 2.71 (d, 3H), 2.00 (s, 3H) 1.78 (m, 6H), 1.21 (m, 2H); MALDI-TOF MS, calc. for (M^+H^+) 1310.4, found 1311.7.

Preparation of ImPyPyPyPyPyPy-G-Ed (**1a**). This compound was synthesized and purified by the general procedures described above to yield 6.7 mg (19%) of ImPyPyPyPyPyPy-G-Ed (**1a**) which was 97% pure. A portion of this material was purified a second time by preparatory HPLC to give pure **1a** (0.8 mg).

Characterization of **1a**. HPLC, r.t. 28.3; UV(H_2O/DMSO)λ_{max}(ε), 246 (35,600), 312 (57,000); ^{1}H NMR (DMSO-d_6) δ 10.48 (s, 1H), 9.99(s, 1H,

PyNH), 9.96 (m, 4H, PyNH), 8.26 (t, 1H, J = 6.3 Hz, G-NH), 8.04 (t, 1H, J = 5.3 Hz, PyCONH-Gly), 7.75-7.67 (br s, 3H, NH_3), 7.40 (s, 1H), 7.28 (d, 1H J = 1.7 Hz), 7.23 (m, 5H), 7.17 (d, 1H, J = 1.7 Hz), 7.08 (m, 4H), 7.05 (d, 1H, J = 1.7 Hz), 6.95 (d, 1H, J = 1.7 Hz), 3.98 (s, 3H), 3.85 (m, 15 H), 3.79 (s, 3H), 3.74 (d, 2H, J = 6.4 Hz, $GlyCH_2$), 3.31 (q, 2H, J = 6.1 Hz, Gly-NH-CH_2), 2.85 (m, 2H, J = 3.0 Hz, CH_2NH_2); MALDI-TOF MS, calc. for M^+H^+ 959.0, found 959.3.

Preparation of ImPyPyPyPyPyPy-G-Dp (**1b**). This compound was synthesized by the general procedures described above to give 8 mg, 24% yield of ImPyPyPyPyPyPy-G-Dp (**1b**). A portion of this material was purified a second time by preparatory HPLC to give pure **1b** (1.2 mg).

Characterization of **1b**. HPLC, r.t. 28.5; UV(H_2O/DMSO)λ_{max}(ϵ), 246 (34,600), 312 (55,300); ^{1}H NMR (DMSO-d_6) δ 10.55 (s, 1H, PyNH), 10.02(s. 1H, PyNH), 10.00 (m, 4H, PyNH), 9.3 (br s, 1H, CF_3COOH), 8.32 (t, 1H, J = 6.2 Hz, Gly-NH), 8.06 (t, 1H, J = 5.9 Hz. PyCONH-Gly), 7.44 (d, 1H, J = 0.6 Hz), 7.31 (d, 1H, J = 1.7 Hz). 7.26 (m, 5H), 7.19 (d, 1H, J = 1.8 Hz). 7.10 (m, 5H), 6.97 (d. 1H, J = 1.7 Hz). 4.01 (s, 3H), 3.87 (m, 15 H), 3.82 (s. 3H), 3.73 (d, 2H, J = 5.5 Hz, $GlyCH_2$), 3.16 (q, 2H. J = 6.2 Hz, Gly-NH-CH_2). 3.03 (q, 2H, J = 5.2 Hz, $CH_2N(CH_3)_2$). 2.74 (d, 6H. J = 4.9 Hz, $CH_2N(CH_3)_2$). 1.77 (quintet, 2H, J = 6.7 Hz, $CH_2CH_2N(CH_3)_2$); MALDI-TOF MS, calc. for 1001.1, found 1000.5.

Preparation of ImPyPyPyPyPyPy-G-Ta (**1c**). This compound was synthesized by the general procedures described above to yield 9.2 mg, (28%) of product **1c**.

Characterization of **1c**. HPLC. r.t. 29.3; UV(H_2O/DMSO)λ_{max}(ϵ), 246 (33. 400), 312 (53,500). ^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1H, PyNH), 9.95

(m, 5H, PyNH), 9.4 (br s, 1H, CF_3COOH), 8.28 (t, 1H, J = 6.1 Hz, Gly-NH), 8.04 (t, 1H, J = 5.1 Hz, PyCONH-Gly), 7.8 (br s, 3H, CH_2NH_3), 7.39, (d, 1H, J = 0.6 Hz), 7.28 (d, 1H, J = 1.2 Hz), 7.23 (m, 5H), 7,17 (d, 1H, J = 1.8 Hz), 7.09 (m, 4H), 7.04 (d, 1H, J = 1.7 Hz), 6.96 (d, 1H, J = 1.6 Hz), 3.98 (s, 3H), 3.85 (m, 15 H), 3.79 (s, 3H), 3.72 (d, 2H, J = 5.2 Hz, $GlyCH_2$), 3.15 (q, 2H, J = 5.0 Hz), 3.11 (m, 4H), 2.80 (m, 2H), 2.74 (d, 3H, J = 2.9 Hz, NCH_3), 1.89 (quintet, 2H, J = 7.4 Hz), 1.77 (quintet, 2H, J = 6.8 Hz); MALDI-TOF MS, calc. for M^+H^+ 1044.2, found 1044.1.

Preparation of ImPyPyPyPyPyPy-G-Ta-EDTA (1d). Synthesized by the general procedures described above to yield 1.1 mg, 32% yield of compound **1d**.

Characterization of 1d. HPLC, r.t. 30.6; UV(H_2O/DMSO)λ_{max}, 246, 312; ^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1H), 9.46 (m, 4H), 7.39 (s, 1H), 7.28 (d, 1H, J = 1.7 Hz), 7.28 (d, 1H, J = 1.6 Hz), 7.24 (d, 1H, J = 1.6 Hz), 7.23 (m, 4H), 7.17 (d, 1H, J = 1.7 Hz), 7.08 (m, 5H), 7.04 (d, 1H, J = 1.6 Hz), 6.95 (d, 1H, J = 1.5 Hz), 3.98 (s, 3H), 3.84 (m, 15H), 3.79 (s, 3H), 3.71 (d, 2H), 3.66 (m, 4H), 3.26 (m, 8H), 3.13 (m, 4H), 2.73 (d, 3H), 2.27 (t, 2H), 1.78 (m, 6H), 1.21 (m, 2H); MALDI-TOF MS, calc. for M^+H^+ 1317.4, found 1318.1.

Preparation of AcImPyPy-γ-PyPyPy-G-Dp (3a). This compound was synthesized by the general procedures set forth above to yield 13.1 mg (30%) of compound **3a**. The only variation was that Boc-γ is activated *in situ*.

Characterization of 3a. HPLC, r.t. 24.0; UV(H_2O/DMSO)$\lambda_{max}(\epsilon)$, 246 (35,900), 312 (48,800); ^{1}H NMR (DMSO-d_6) δ 10.23 (s, 1H), 9.98 (s, 1H), 9.32 (s, 1H), 9.90 (m, 2H), 9.84 (s, 1H), 9.2 (br s, 1H), 8.27 (t, 1H J = 5.0 Hz), 8.05 (m, 2H), 7.41 (s, 1H), 7.25 (d, 1H J = 1.2 Hz), 7.22 (m, 2H), 7.16 (m, 2H), 7.12 (d, 1H, J=1.7), 7.05 (d, 1H, J = 1.5 Hz), 6.94 (d, 1H, J = 1.6 Hz).

6.89 (d, 1H, J = 1.7 Hz) 6.87 (d, 1H, J = 1.6 Hz), 3.93 (s, 3H), 3.83 (s, 3H), 3.82 (m, 6H), 3.81 (s, 3H), 3.79 (s, 3H), 3.71 (d, 2H, J = 5.1 Hz), 3.19 (m, 2H, J=5.8), 3.12 (m, 2H J = 5.0 Hz), 3.01 (m, 2H, J = 4.2 Hz), 2.74 (d, 6H, J = 4.6 Hz), 2.26 (m 2H, J = 4.6 Hz), 2.00 (s, 3H), 1.75 (m, 4H); MALDI-TOF MS, calc. for M^+H^+ 1021.1, found 1021.6.

Preparation of AcImPyPy-γ-PyPyPy-G-Ta (3b). This compound was synthesized by the general procedures set forth above to yield 9.2 mg (31%) of product **3b**.

Characterization of **3b**. HPLC, r.t. 24.9; UV(H_2O/DMSO)λ_{max}(ϵ), 246 (37,400), 312 (50,500); ^{1}H NMR (DMSO-d_6) δ 10.24 (s, 1H), 9.98 (s, 1H), 9.94 (s, 1H), 9.91 (m, 2H), 9.85 (s, 1H), 9.7 (br s, 1H), 8.28 (t, 1H, J = 5.2 Hz), 8.05 (m, 2H), 7.86 (br s, 3H), 7.42 (d, 1H, J = 1.6 Hz), 7.26 (d, 1H, J = 1.7 Hz), 7.22 (m, 2H), 7.17 (m, 2H), 7.13 (d, 1H, J = 1.7 Hz), 7.06 (d, 1H, J = 1.7 Hz), 6.95 (d, 1H, J = 1.7 Hz), 6.90 (d, 1H, J = 1.7 Hz), 6.88 (d, 1H, J = 1.7 Hz). 3.94 (s, 3H), 3.84 (s, 6H), 3.82 (s, 3H), 3.80 (s, 6H), 3.72 (s, 3H), 3.14 (m, 6H), 3.08 (m, 2H), 2.85 (m, 2H), 2.75 (d, 3H, J = 4.2 Hz), 2.72 (t, 2H, J = 6.8 Hz), 2.01 (s, 3H), 1.90 (m, 2H), 1.75 (m, 4H); MALDI-TOF-MS, calc. for M^+H^+ 1064.2, found 1064.5.

Preparation of AcImPyPy-γ-PyPyPy-G-Ta-EDTA (3c). Synthesized by the general procedures described above to yield 3.2 mg (41%) of compound **3c**.

Characterization of **3c**. HPLC, r.t. 24.3; UV(H_2O DMSO)λ_{max}, 246, 312; ^{1}H NMR (DMSO-d_6) δ 10.32 (s, 1H), 9.97 (s, 1H), 9.93 (s, 1H), 9.90 (m, 2H), 9.84 (s, 1H), 8.40 (t, 1H), 8.27 (t, 1H), 8.05 (m, 2H), 7.41 (s, 1H), 7.25 (d, 1H, J = 1.6 Hz), 7.20 (m, 2H), 7.16 (m, 2H), 7.11 (d, 1H, J = 1.6 Hz), 7.05 (d, 1H, J = 1.7 Hz), 6.94 (d, 1H, J = 1.7 Hz), 6.86 (d, 1H, J = 1.7 Hz), 3.92

(s, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.78 (m, 6H), 3.72 (d, 2H, J = 5.5 Hz), 3.66 (m, 4H), 3.40 (m, 10H), 3.15 (m, 6H), 2.73 (d, 3H, J = 4.2 Hz), 2.27 (t, 2H, J = 6.9 Hz), 2.03 (s, 3H), 1.78 (m, 6H), 1.23 (m, 2H); MALDI-TOF-MS, calc. for M^+H^+ 1339.4, found 1340.

Preparation of ImImPy-γ-PyPyPy-G-Dp **(4d)**. Synthesized by the general procedures described above to yield 8.9 mg (30%) ImImPy-γ-PyPyPy-G-Dp **(4d)**.

Characterization of **4d**. HPLC, r.t. 24.6; UV(H_2O/DMSO)$\lambda_{max}(\epsilon)$, 246 (37,600), 312 (50,700); ^{1}H NMR (DMSO-d_5) δ 10.30 (s, 1H), 10.28 (s, 1H), 9.93 (s, 1H), 9.90 (s, 1H), 9.84 (s, 1H), 9.33 (s, 1H), 9.28 (br s , 1H), 8.05 (t, 1H, J = 5.1), 8.08 (t, 1H), 8.02 (t, 1H), 7.56 (s, 1H), 7.50 (s, 1H), 7.21 (m, 3H), 7.16 (d, 1H, J = 1.2 Hz), 7.05 (d, 1H, J = 1.1 Hz), 7.00 (d, 1H, J = 1.4 Hz), 6.94 (d, 1H, J = 1.4 Hz), 6.87 (d, 1H, J = 1.2 Hz), 3.99 (s, 3H), 3.97 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.71 (d, 2H, J = 4.2 Hz), 3.20 (m, 2H), 3.14 (m, 2H), 3.01 (m, 2H), 2.75 (d, 6H, J = 3.2 Hz), 2.27 (t, 2H, J = 7.2 Hz), 2.03 (s, 3H), 1.76 (m, 4H); IR (neat) 3260 (m), 2927 (w) 2332 (w), 1666 (s), 1531 (s), 1449 (m), 1396 (w), 1196 (w), 1126 (w); MALDI-TOF-MS, calc. for $C_{47}H_{60}N_{18}O_9$ M^+H^+ 1022.1, found 1022.4.

AcImImPy-γ-PyPyPy-G-Dp **(4a)**. Synthesized by the general procedures described above to yield 8.9 mg (30%) of compound **4a**.

Characterization of **4a**. HPLC, r.t. 24.1; UV(H_2O/DMSO)$\lambda_{max}(\epsilon)$, 246 (37,600), 312 (50,700); ^{1}H NMR (DMSO-d_6) δ 10.30 (s, 1H), 10.28 (s, 1H), 9.93 (s, 1H), 9.90 (s, 1H), 9.84 (s, 1H), 9.33 (s, 1H), 9.28 (br s, 1H), 8.05 (t, 1H, J = 5.1 Hz), 8.08 (t, 1H), 8.02 (t, 1H), 7.56 (s, 1H), 7.50 (s, 1H), 7.21 (m, 3H), 7.16 (d, 1H, J = 1.2 Hz), 7.05 (d, 1H, J = 1.1 Hz), 7.00 (d, 1H, J = 1.4

Hz), 6.94 (d, 1H, J = 1.4 Hz), 6.87 (d, 1H, J = 1.2 Hz), 3.99 (s, 3H), 3.97 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.71 (d, 2H, J = 4.2 Hz), 3.20 (m, 2H), 3.14 (m, 2H), 3.01 (m, 2H, J = 6.2 Hz), 2.75 (d, 6H, J = 3.2 Hz), 2.27 (t, 2H, J = 7.2 Hz), 2.03 (s, 3H), 1.76 (m, 4H); MALDI-TOF-MS, calc. for M^+H^- 1022.1, found 1022.7.

AcImImPy-γ-PyPyPy-G-Ta (**4b**). Synthesized by the general procedures described above to yield 7.4 mg (25%) of compound **4b**.

Characterization of **4b**. HPLC, r.t. 23.8; UV(H_2O/DMSO)λ_{max}(ϵ), 246 (37,000), 312 (50000); ^{1}H NMR (DMSO-d_6) δ 10.31 (s, 1H), 10.29 (s, 1H), 9.93 (s, 1H), 9.90 (s, 1H), 9.84 (s, 1H), 9.34 (s, 1H), 8.31 (t, 1H, J = 5.0 Hz), 8.08 (m, 2H), 7.80 (br s, 3H), 7.56 (s, 1H), 7.50 (s, 1H), 7.20 (m, 3H), 7.15 (d, 1H, J = 1.2 Hz), 7.06 (d, 1H, J = 1.2 Hz), 7.00 (d, 1H, J = 1.3 Hz), 6.95 (d, 1H, J = 1.2 Hz), 6.88 (d, 1H, J = 1.3 Hz), 3.98 (s, 3H), 3.96 (s, 3H), 3.82 (s, 3H), 3.82 (s, 3H), 3.79 (m, 6H), 3.71 (d, 2H, J = 4.9 Hz), 3.15 (m, 6H), 3.06 (m, 2H, J = 4.7 Hz), 2.84 (m, 2H, J = 4.9 Hz), 2.74 (d, 3H, J = 4.2 Hz), 2.27 (t, 2H), 2.02 (s, 3H), 1.89 (m, 2H), 1.75 (m, 4H); MALDI-TOF-MS, calc. for M^+H^+ 1065.2, found 1065.2.

Preparation of AcImImPy-γ-PyPyPy-G-Ta-EDTA (**4c**). Synthesized by the general procedures described above to yield 2.1 mg (35%) of product **4c**.

Characterization of **4c**. HPLC, r.t. 23.8; UV(H_2O/DMSO)λ_{max}, 246, 312; ^{1}H NMR (DMSO-d_6) δ 10.31 (s, 1H), 10.29 (s, 1H), 9.93 (s, 1H), 9.90 (s, 1H), 9.84 (s, 1H), 9.33 (s, 1H), 9.21 (br s, 1H), 8.37 (t, 2H, J = 4.6 Hz), 8.28 (t, 1H, J = 5.2 Hz), 8.09 (t, 1H, J = 5.2 Hz), 8.03 (t, 1H, J = 5.4 Hz), 7.6 (s, 1H), 7.5 (s, 1H), 7.21 (m, 3H), 7.15 (d, 1H, J = 0.8 Hz), 7.06 (d, 1H, J = 1.0 Hz), 7.00 (d, 1H, J = 1.2 Hz), 6.95 (d, 1H, J = 1.1 Hz), 6.88 (d, 1H, J = 1.2 Hz),

3.99 (s, 3H), 3.96 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 3.79 (m, 6H), 3.71 (d, 2H, J = 5.0 Hz), 3.66 (m, 4H), 3.25 (m, 10H), 3.15 (m, 6H), 2.72 (d, 3H, J = 4.4 Hz), 2.47 (t, 2H), 2.00 (s, 3H), 1.77 (m, 6H), 1.27 (m, 2H); MALDI-TOF-MS, calc. for M^+H^+ 1338.4, found 1338.6.

Preparation of AcPyPyPy-γ-ImImPy-Gly-Dp (5a). Synthesized by the general procedures described above to yield 9.9 mg (37%) of compound **5a**.

Characterization of **5a**. HPLC, r.t. 23.8; UV(H_2O/DMSO)$\lambda_{max}(\epsilon)$, 246 (41,800), 312 (56,400); ^{1}H NMR (DMSO-d_6) δ 10.34 (s, 1H), 10.33 (s, 1H), 9.90 (m, 1H), 9.83 (s, 1H), 9.35 (s, 1H), 9.29 (br s, 1H), 8.29 (t, 1H, J = 5.3 Hz), 8.03 (m, 2H), 7.56 (s, 1H), 7.53 (s, 1H), 7.27 (d, 1H, J = 1.0 Hz), 7.22 (d, 1H, J = 7.16 Hz), 7.16 (d, 1H, J = 0.9 Hz), 7.13 (d, 1H, J = 1.0 Hz), 7.03 (m, 2H), 6.88 (d, 1H, J = 1.1 Hz), 6.84 (d, 1H, J = 1.0 Hz), 3.98 (s, 3H), 3.97 (s, 3H), 3.92 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.72 (s, 3H), Gly CH_2 was covered by water, 3.20 (m, 2H, J = 5.6 Hz), 3.12 (m, 2H, J = 5.9 Hz), 3.02 (quintet, 2H, J = 4.1 Hz), 2.74 (d, 6H, J = 4.4 Hz), 2.36 (t, 2H, J = 7.0 Hz), 1.95 (s, 3H), 1.77 (m, 4H); MALDI-TOF-MS, calc. for M^-H^+ 1022.1, found 1022.4.

Preparation of AcPyPyPy-γ-ImImPy-G-Ta (**5b**). Synthesized by the general procedures described above to yield 8.2 mg (27%) of compound **5b**.

Characterization of **5b**. HPLC, r.t. 23.6; UV(H_2O/DMSO)$\lambda_{max}(\epsilon)$, 246 (39,300), 312 (53,100); ^{1}H NMR (DMSO-d_6) δ 10.38 (s, 1H), 10.34 (s, 1H), 9.92 (m, 2H), 9.85 (s, 1H), 9.35 (s, 1H), 8.33 (t, 1H), 8.07 (m, 2H), 7.82 (br s, 1H), 7.57 (s, 1H), 7.54 (s, 1H), 7.28 (d, 1H, J = 1 Hz), 7.23 (d, 1H, J = 1 Hz), 7.17 (d, 1H, J = 1 Hz), 7.14 (d, 1H, J = 1 Hz), 7.14 (d, 1H, J = 1.3 Hz), 7.04 (m, 2H), 6.89 (d, 1H, J = 1 Hz), 6.84 (d, 1H, J = 1

Hz), 3.99 (s, 3H), 3.97 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.71 (d, 2H, J = 5.2 Hz), 3.26 (m, 2H), 3.14 (d, 2H, J = 5.1 Hz), 3.05 (m, 2H), 2.83 (q, 2H, J = 5.6 Hz), 2.74 (d, 3H, J = 4.3 Hz), 2.39 (m, 2H), 1.96 (s, 3H), 1.88 (q, 2H, J = 6.6 Hz), 1.78 (m, 2H); MALDI-TOF-MS, calc. for M^+H^+ 1065.2, found 1065.9.

Preparation of AcPyPyPy-γ-ImImPy-G-Ta-EDTA (5c).

Synthesized by the general procedures described above to yield 3.1 mg (37%) of **5c**.

Characterization of 5c. HPLC, r.t. 24.0; UV(H_2O/DMSO)$\lambda_{max}(\epsilon)$, 246, 312; ^{1}H NMR (DMSO-d_5) δ 10.37 (s, 1H), 10.34 (s, 1H), 9.91 (m, 2H), 9.84 (s, 1H), 9.37 (s, 1H), 8.38 (t, 1H), 8.32 (t, 1H), 8.06 (m, 2H), 7.57 (s, 1H), 7.53 (s, 1H), 7.27 (s, 1H), 7.22 (s, 1H), 7.17 (s, 1H), 7.14 (s, 1H), 7.04 (m, 2H), 6.88 (s, 1H), 6.85 (s, 1H), 3.99 (s, 3H), 3.96 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 3.79 (m, 6H), 3.71 (d, 2H), 3.64 (m, 4H), 3.25 (m, 10H), 3.15 (m, 6H), 2.72 (d, 3H), 2.50 (t, 2H), 1.95 (s, 3H), 1.79 (m, 6H), 1.22 (m, 2H); MALDI-TOF-MS, calc. for M^-H^+ 1338.4, found 1339.1.

Preparation of EDTA-γ-ImPyPy-β-PyPyPy-G-Dp. The polyamide ImPyPy-β-PyPyPy-G-Dp, synthesized by the general procedures described above, was modified with the dianhydride of EDTA in DMSO/NMP at 55°C for 10 minutes, the anhydride was then opened with 0.1M NaOH (20 minutes), and the reaction mixture purified by reverse phase preparatory HPLC to provide the EDTA modified polyamide.

Preparation of ImPyPy-G-PyPyPy-β-Dp. The polyamide was prepared as described above to yield a white powder. (12.3 mg, 42% recovery). HPLC, r.t. 25.5; UV(H_2O/DMSO)λ_{max} (ϵ), 246 (39,500), 312 (52,000) nm; ^{1}H NMR (DMSO-d_6) δ 10.46 (s, 1H), 9.96 (s, 1H), 9.90 (s, 1H),

9.88 (m, 2H), 9.21 (br s, 1H) 8.27 (t, 1H, *J*=452.5 Hz), 8.06 (m, 2H), 7.39 (s, 1H), 7.28 (d, 1 *H*, *J*=1.6 Hz), 7.23 (d, 1H, *J*=1.7 Hz), 7.20 (d, 1H, *J*=1.5 Hz), 7.15 (m, 3H), 7.04, (m, 2H), 7.03 (d, 1H, *J*=1.6 Hz), 6.94 (d, 1H, *J*=1.7 Hz), 6.92 (d, 1H, *J*=1.4 Hz), 3.98 (s, 3H), 3.88 (d, 2H), 3.83 (s, 3H), 3.82 (m, 6H), 3.79 (s, 3H), 3.78 (s, 3H), 3.36 (q, 2H, *J*=5.3 Hz), 3.09 (q, 2H, *J*=6.0 Hz), 2.99 (m, 2H), 2.75 (t, 2H, *J*=5.2 Hz), 2.72 (d, 6H, *J*=4.8 Hz), 2.30 (t, 2H, *J*=6.3 Hz), 1.72 (quintet, 2H, *J*=5.7 Hz); MALDI-TOF MS 950.06; FABMS *m/e* 949.462 (M + H 949.455 calc. for $C_{45}H_{57}N_{16}O_8$).

Example 5. Symmetric Anhydride Activation of Pyrrole. In a typical symmetric anhydride procedure (0.25 mmol synthesis cycle), the resin was washed with 5% DIEA/CH_2Cl_2. No DIEA should be present, however, at the start of the coupling reaction. Boc-pyrrole-COOH (514 mg, 2 mmol) was slurried in 3 ml dichloromethane, DCC (406 mg, 2 mmol) was then added upon which time the white slurry turned clear. After three minutes, dimethylaminopyridine (DMAP) (101 mg, 1 mmol) was added and the solution was stirred, filtered and added to the reaction vessel containing the resin. The coupling was allowed to proceed for 2 hours, 355 μl DIEA was then added and the reaction allowed to proceed for an additional hour.

Example 6. Picric Acid Test. In order to monitor the progress of the reactions 8-10 mg samples were periodically removed from the deprotection reaction mixtures and evaluated using picric acid titration. The 10 mg sample was washed with dichloromethane, 5% TEA/CH_2Cl_2, and dichloromethane, and dried either at 50°C or by aspiration. A sample of about 5 mg of the dried resin was weighed into a disposable polypropylene filter, successively washed using

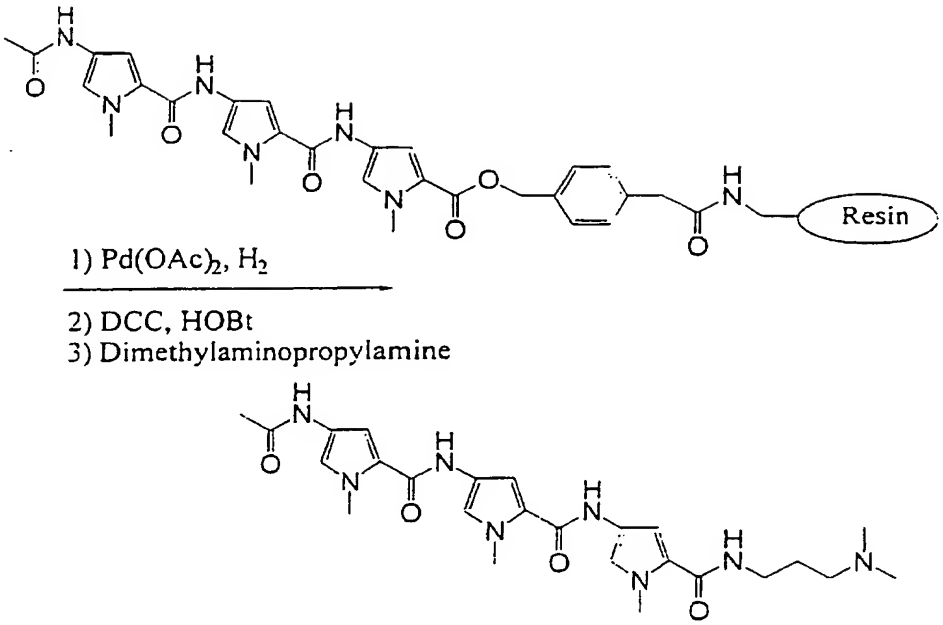
gravity filtration with approximately: 5 ml dichloromethane, 5 ml 0.1M picric acid/dichloromethane and 50 ml dichloromethane to carefully remove any excess picric acid, the picric acid salt eluted with 5% DIEA/CH_2Cl_2 (3 x 500 µl). The DIEA/CH_2Cl_2 wash was collected and diluted with 4 ml MeOH and the absorbance measured at 358 nm.

Example 7. Stepwise HPLC analysis. Approximately 2 mg of a resin sample was placed in a 1.5 ml polypropylene tube, 40 µl of DMF was added and the resin was allowed to stand for 10 minutes. 40 µl of dimethylaminopropylamine was then added and the mixture was vortexed, briefly centrifuged, and heated at 37°C for 12 hours. After 12 hours the solutions were again vortexed, centrifuged, and a 10 µl aliquot was taken, diluted with 90 µl water and analyzed by analytical HPLC under standard conditions at 254 nm.

Example 8. Cleavage of the Polyamide from the Resin using $Pd(OAc)_2$.

Scheme 17 illustrates a general method for cleaving the synthesized polyamide from the resin. The acetylated tripyrrole AcPyPyPy-PAM-resin is used for purposes of illustration.

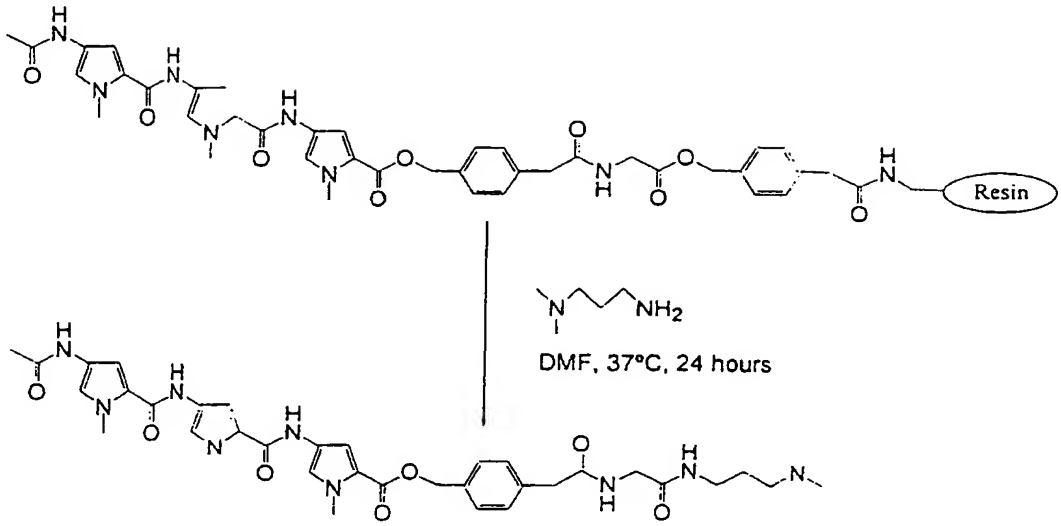
SCHEME 17



The PAM or BAM pyrrole resin was treated with $Pd(OAc)_2$ in DMF under a pressurized atmosphere of hydrogen (100 psi, 8 hours). The palladium black was filtered and the pyrrole acid activated with DCC.HOBt and reacted with a large excess of dimethylaminopropylamine to give the HPLC purified acetylated tripyrrole in 5% overall yield. HPLC and NMR are consistent with that of an authentic standard synthesized by solution phase methods by Wade *et al.* (1992) J. Am. Chem. Soc. 114:8783-8794.

Example 9. Cleavage of the Polyamide from the G-PAM-resin. Scheme 18 illustrates a general method for cleaving the synthesized polyamide from the G-PAM-resin. The acetylated tripyrrole AcPyPyPy-PAM-G-PAM-resin is used for purposes of illustration.

SCHEME 18



180 mg (29 μmole) of AcPyPyPy-PAM-G-PAM-resin was treated with 1.5 ml DMF followed by 1.5 ml dimethylaminopropylamine and the reaction mixture shaken for 12 hours, and purified by preparatory HPLC to give AcPyPyPy-PAM-G-Dp in 49% yield. ^{1}H NMR (DMSO-d_5) δ 9.90 (m, 2H), 9.83 (s, 1H), 9.3 (br s, 1H), 8.37 (t, 1H, J = 5.7 Hz), 8.05 (t, 1H, J = 5.8 Hz), 7.44 (d, 1H, J = 1.7 Hz), 7.32 (q, 4H, J = 8.2 Hz), 7.20 (d, 1H, J = 1.7 Hz), 7.13 (d, 1H, J = 1.7 Hz), 7.04 (d, 1H, J = 1.7 Hz), 6.95 (d, 1H, J = 1.9 Hz), 6.83 (d, 1H, J = 1.8 Hz), 5.19 (s, 2H), 3.82 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.63 (d, 2H, J = 6.1 Hz), 3.48 (s, 2H), 3.11 (q, 2H, J = 6.1 Hz), 2.96 (m, 2H), 2.67 (d, 6H, J = 4.8 Hz), 1.95 (s, 3H), 1.71 (quintet, 2H, J = 7.4 Hz). A failure sequence was also isolated from the reaction mixture in 25% yield. ^{1}H NMR (DMSO-d_6) δ 9.91 (m, 2H), 9.80 (s, 1H), 9.3 (br s, 1H), 8.40 (t, 1H, J = 5.7 Hz), 8.08 (t, 1H, J = 5.8 Hz), 7.44 (d, 1H, J = 1.7 Hz), 7.38 (q, 4H, J = 8.4 Hz), 7.15 (d, 1H, J = 1.7 Hz), 6.96 (d, 1H, J = 1.8 Hz), 6.85 (d, 1H, J = 1.7 Hz), 5.15

(s, 2H), 3.84 (s, 3H), 3.82 (s, 3H), 3.69 (d, 2H, J = 5.4 Hz), 3.51 (s, 2H), 3.19 (m, 2H), 3.04 (m, 2H), 2.74 (d, 6H, J = 4.2 Hz), 1.97 (s, 3H), 1.77 (m, 2H).

Example 10. Quantitative DNase I footprint titrations. All reactions were executed in a total volume of 40 µL. A polyamide stock solution (H_2O containing no polyamide was used for reference reactions) was added to an assay buffer containing radio labeled restriction fragment (15,000 cpm), affording final solution conditions of 10 mM Tris HCl, 10 mM KCl, 10 mM $MgCl_2$, 5 mM $CaCl_2$, pH 7.0 and (i) 0.1 nM - 1 µM polyamide, for all polyamides except ImPyPy-β-PyPyPy-Dp and ImPyPy-β-PyPyPy-G-Dp, (ii). .01 nM - 0.1 µM polyamide for ImPyPy-β-PyPyPy-Dp and ImPyPy-β-PyPyPy-G-Dp. The solutions were allowed to equilibrate for 5 hours at 22°C. Footprinting reactions were initiated by the addition of 4 µL of DNase I stock solution (at the appropriate concentration to give 55% intact labeled DNA) containing 1 mM dithiothreitol. The reactions were allowed to proceed for approximately seven minutes at 22°C. After seven minutes the reactions were by the addition of 10 µL of a solution containing 1.25 M NaCl, 100 mM EDTA, and 0.2 mg/ml glycogen, and ethanol precipitated. The reactions were resuspended in 1X TBE/80% formamide loading buffer, denatured at 85°C for 10 minutes, placed on ice, and loaded onto an 8% polyacrylamide gel (5% cross-link, 7 M urea). The reaction products were separated by electrophoresis in 1X TBE at 2000 V. Gels were dried and exposed to a storage phosphor screen (Molecular Dynamics). Figure 15 depicts the storage phosphor autoradiogram of 8% denaturing polyacrylamide gels used to separate the fragments generated by DNase I digestion in quantitative footprint titration experiments: lanes 1-2, A and G sequencing lanes; lanes 3 and 21, DNase I

digestion products obtained in the absence of polyamide; lanes 4-20, DNase I digestion products obtained in the presence of 0.1 nM (0.01 nM), 0.2 nM, (0.02 nM), 0.5 nM (0.05 nM), 1 nM (0.1 nM), 1.5 nM (0.15 nM), 2.5 nM (0.25 nM), 4 nM (0.4 nM), 6.5 nM (0.65 nM), 10 nM (1 nM), 15 nM (1.5), 25 nM (2.5 nM), 40 nM (4 nM), 65 nM (6.5 nM), 100 nM (10 nM), 200 nM (20 nM), 500 nM (10 nM), 1 μM (0.1 μM) concentrations were used for polyamides ImPyPy-β-Ala-PyPyPy-Dp and ImPyPy-β-Ala-PyPyPy-Dp only are in parentheses); lane 22, intact DNA. The five binding sites that were analyzed by quantitative footprint titration experiments are indicated on the right sides of the autoradiogram.

Data from the footprint titration gels were obtained using a Molecular Dynamics 400S PhosphorImager followed by quantitation using ImageQuant software (molecular Dynamics).

Background-corrected volume integration of rectangles encompassing the footprint sites and a reference site at which DNase I reactivity was invariant across the titration generated values for the site intensities (I_{site}) and the reference intensity (I_{ref}). The apparent fractional occupancy (q_{app}) of the sites were calculated using the equation:

$$\theta_{app} = 1 - \frac{I_{site}/I_{ref}}{I^{o}_{site}/I^{o}_{ref}} \qquad (1)$$

where I^{o}_{site} and I^{o}_{ref} are the site and reference intensities, respectively, from a control lane to which no polyamide was added.

The ($[L]_{tot}$, θ_{app}) data points were fit to a general Hill equation (eq) by minimizing the difference between θ_{app} and θ_{fit}:

$$\theta_{fit} = \theta_{min} + \theta_{max} + \theta_{min}) \frac{Ka^{n}[L]_{tot}^{n}}{1 + Ka^{n}[L]_{tot}^{n}} \quad (2)$$

where $[L]_{tot}$ is the total polyamide concentration, K_a is the apparent first-order association constant, and θ_{min} and θ_{max} are the experimentally determined site saturation values when the site is unoccupied or saturated, respectively. The data were fit using a nonlinear least-squares fitting procedure with K_a, n, θ_{min} as the adjustable parameters. In cases for which the best-fit value of n was ≥ 1.5, the data were fit with n = 2, with K_a, θ_{max}, and θ_{min} as the adjustable parameters. The binding isotherms were normalized using the following equation:

$$\theta_{norm} = \frac{\theta_{app} - \theta_{min}}{\theta_{max} - \theta_{min}} \quad (3)$$

Three sets of data were used in determining each association constant.

At higher concentrations of polyamide (>~ 0.1 μM for ImPyPy-β-PyPyPy-Dp and ImPyPy-β-PyPyPy-Dp, and > 1 μM for the other six polyamides), the reference sites become partially protected due to non-specific DNA-binding, resulting in low θ_{app} values. For this reason, higher concentrations were not used. As a consequence, association constants for sites that are not saturated or nearly saturated at the highest concentration of polyamide used can be determined only approximately. The method for determining association constants used here involved the assumption that $[L]_{tot} = [L]_{free}$ where $[L]_{free}$ is the concentration of polyamide free in solution (unbound). For very high association constants this assumption becomes invalid, resulting in underestimated association constants. In these experiments, the concentration of DNA is estimated to be 50 pM. As a

consequence, association constants of 2 x 10^9 M^{-1} and 5 x 10^9 M^{-1} will be underestimated by approximately 90% and 80%, respectively.

Example 11. Preparation of Dimers

Preparation of Ethyl 4-[[(tert-butyloxy)carbonyl]amino]-1-methylpyrrole-2-(4-carboxamido-1-methyl-imidazole)-2-carboxylate (44). To a solution of 4-[[(*tert*-butyloxy)carbonyl]amino]-1-methylpyrrole-2-carboxylic acid (8 g, 33 mmol) in 20 ml DMF was added 1.2 eq HOBt (5.3 g, 39 mmol) followed by 1.2 eq DCC (8 g, 39 mmol). The solution was stirred for 24 hours, after which the DCU byproduct was removed by filtration. Separately, to a solution of ethyl 4-nitro-1-methylimidazole-2-carboxylate (8 g, 40 mmol) in 20 ml DMF was added Pd/C catalyst (10%, 1 g), and the mixture hydrogenated in a Parr bom apparatus (500 psi H_2) for 2 hours. The catalyst was removed by filtration through celite, and the filtrate immediately added to the -OBt ester solution, an excess of DIEA (10 ml, 110 mmol) added, and the mixture stirred at 37°C for 48 hours. The reaction mixture was added dropwise to a stirred solution of ice water and the resulting precipitate collected by vacuum filtration and dried *in vacuo* to yield a brown powder. (12.3 g, 94% yield). ^{1}H NMR (DMSO-d_6) δ 10.7 (s, 1H), 9.2 (s, 1H), 7.6 (s, 1H), 6.8 (d, 1H), 4.3 (q, 2H), 3.9 (s, 3H), 3.7 (s, 3H), 1.5 (s, 9H), 1.3 (t, 3H).

Preparation of 4-[[(tert-butyloxy)carbonyl]amino]-1-methylpyrrole-2-(4-carboxamido-1-methylimidazole)-2-carboxylic acid (45) . To a solution of ethyl 4 [[tert-butyloxy)carbonyl]amino]-1-methylpyrrole-2-(4-carboxamido-1-methylimidazole)-2-carboxylate (44) (5 g, 12.7 mmol) in 50 ml methanol was added 50 ml 1M KOH and the reaction was allowed to stir for 6 hours at 37°C. Excess methanol was removed *in vacuo* and the resulting

solution acidified by the addition of 10% potassium bisulfate. The resulting precipitate was collected by vacuum filtration and dried *in vacuo* to yield a brown powder. (4.4 g, 89% yield). ^{1}H NMR (DMSO-d_6) δ 10.9 (s, 1H), 8.9 (s, 1H), 7.6 (s, 1H), 7.3 (d, 1H), 6.9 (d, 1H), 4.1 (s, 3H), 3.9 (s, 3H), 1.4 (s, 9H).

Preparation of Ethyl γ-[[tert-butyloxy)carbonyl]amino]-butyric acid -(4-carboxamido-1-methylimidazole)-2-carboxylate (46). To a solution of Boc-γ-aminobutyric acid (10 g, 49 mmol) in 40 ml DMF was added 1.2 eq HOBt (7.9 g, 59 mmol) followed by 1.2 eq DCC (11.9 g, 59 mmol). The solution was stirred for 24 hours, and the DCU byproduct was removed by filtration. Separately, to a solution of ethyl 4-nitro-1-methylimidazole-2-carboxylate (9.8 g. 49 mmol) in 20 ml DMF was added Pd C catalyst (10%, 1 g), and the mixture was hydrogenated in a Parr bom apparatus (500 psi H_2) for 2 hours. The catalyst was removed by filtration through celite and the filtrate was immediately added to the -OBt ester solution. An excess of DIEA (15 ml) was then added and the reaction was stirred at 37°C. After 48 hours the reaction mixture was added dropwise to a stirred solution of ice water and the resulting precipitate collected by vacuum filtration and dried *in vacuo* to yield a brown powder. (9.4 g, 54% yield). ^{1}H NMR (DMSO-d_6) δ 10.6 (s, 1H), 7.6 (s, 1H), 6.9 (t, 1H), 4.2 (q, 2H), 3.9 (s, 3H), 2.9 (q, 2H), 2.3 (t, 2H), 1.4 (s, 9H), 1.3 (t, 3H).

Preparation of γ-[[(tert-butyloxy)carbonyl]amino]-butyric acid -(-4-carboxamido-1-methyl-pyrrole)-2-carboxylate (47). To a solution of ethyl γ-[[(tert-butyloxy)carbonyl]amino]-butyric acid -(4-carboxamido-1-methyl)-2-carboxylate (5 g. 14.1 mmol) in 50 ml methanol was added 50 ml 1M KOH and the resulting mixture was allowed to stir for 6 hours at 37°C. Excess methanol was removed *in vacuo* and the resulting solution acidified by the

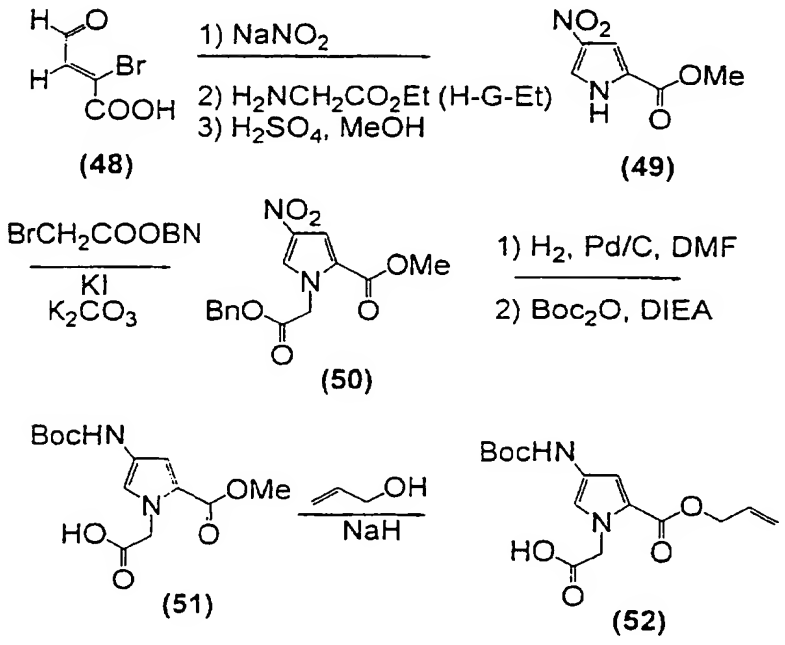
addition of 10% potassium bisulfate. The resulting precipitate was collected by vacuum filtration and dried *in vacuo* to yield a brown powder. (4.1 g, 91% yield). ^{1}H NMR (DMSO-d_5) δ 10. 6 (s, 1H), 7.6 (s, 1H), 6.8 (t, 1H), 3.9 (s, 3H), 2.8 (q, 2H), 2.3 (q, 2H), 1.7 (t, 2H), 1.5 (s, 9H).

Activation of Boc-X-Im-COOH. Boc-γ-Im-COOH or Boc-Py-Im-COOH (100 mg, about 0.3 mmol) and HBTU (2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluoro phosphate) (118 mg, 0.3 mmol) were dissolved in 500 μl DMF, 100 μl DIEA was added and the solution allowed to stand for 3 minutes.

Example 12. Preparation of Allyl Protected Monomers

Scheme 19 illustrates a general method for the preparation of allyl protected monomers for use in preparation of cyclic polyamides.

SCHEME 19



Methyl-4-nitropyrrole-2-carboxylic acid (49). To a solution of sodium nitrite (714 g, 10.2 mol) in 700 ml of water at 50°C ± 5°C was added over a period of 3 hours a solution of mucobromic acid (48) (700 g, 2.7 mol) in 700 ml of warm ethanol. The reaction was stirred for 15 minutes, cooled to 0°C, and 700 ml of ethanol was added. The resulting orange precipitate was collected by vacuum filtration and dried *in vacuo* to yield sodium nitromalondialdehyde monohydrate (305 g, 1.9 mol) which was used in the next step without further purification. Glycine ethyl ester (300 g, 2.9 mol) and nitromalondialdehyde monohydrate (305 g, 1.9 mol) were placed in a 12 l round bottom flask and slurried with a mechanical stirrer in 560 ml of methanol and 280 ml of water. Sodium hydroxide (800 g, 20 mol) dissolved in 1.6 l water was added to the solution at a rate which maintained the temperature at 50°C. The reaction was cooled to 0°C with an ice bath and neutralized to pH 0 with 1.7 l HCl (conc.) while maintaining the temperature below 10°C. A black precipitate was removed by filtration through Celite and the product was extracted with 20 liters of ethyl acetate. The organic layer was dried with sodium sulfate and concentrated *in vacuo* to provide 4-nitropyrrole-2-carboxylic acid (140 g, 0.9 mol) as a brown solid which was used without further purification. To a solution of 4-nitropyrrole-2-carboxylic acid (140 g, 0.9 mol) dissolved in 180 ml of methanol was added 8 ml of concentrated sulfuric acid. The solution was refluxed for 15 hours, cooled to 0°C and 90 ml of water was added. The solution was then allowed to stand at -20°C for 2 days. The resulting light brown crystals were collected by vacuum filtration to yield pure methyl 4-nitropyrrole-2-carboxylate (49) (105 g, 0.62 mol) in 23%

overall yield. ^{1}H NMR (DMSO-d_6) δ 14.2 (br s, 1H), 7.74 (d, 1H, *J*=1.6 Hz), 7.35 (d, 1H, *J*=1.7 Hz), 3.73 (s, 3H).

Preparation of 1-(Benzyloxycarbonylmethyl)-2-carboxy-4-nitropyrrole methyl ester (**50**). To a solution of methyl 4-nitropyrrole-2-carboxylate (**49**) (8.1 g, 48 mmol) dissolved in 100 ml of acetone was added potassium carbonate (19.5 g, 141 mmol) and potassium iodide (7.2 g, 43.4 mmol), followed by 2-benzylbromoacetate (18.9 ml). The solution was refluxed for 2 hours, an additional 5 ml of 2-benzylbromoacetate was added and the solution was refluxed for an additional 2 hours. The reaction mixture was concentrated *in vacuo*, partitioned between 300 ml of water and 300 ml of dichloromethane and extracted with dichloromethane (2 x 100 ml). The extracts were combined, dried over sodium sulfate and concentrated *in vacuo*. The resulting oil was purified by flash chromatography (4:1 hexane:ethyl acetate) to yield 910.4 g (32.7 mmol, 68%) of the diester (**50**). ^{1}H NMR (DMSO-d_6) δ 8.30 (d, 1H, *J*=1.9 Hz), 7.35 (d, 1H, *J*=1.9 Hz). 7.35 (s, 5H), 5.27 (s, 2H), 5.19 (s, 2H), 3.17 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 168.1, 160.3, 135.2, 130.2, 129.0. 128.8, 128.6, 123.1. 112.1., 67.1, 52.5, 51.5; FABMS, found 318.085, calc. 318.085.

Preparation of methyl 1-(carboxymethyl)-4-[[*tert*-butyloxy)carbonyl]amino]-2-carboxylate (**51**). To a solution of diester (**50**) (4.3 g, 13.5 mmol) in 80 ml of DMF was added Pd/C catalyst (10%, 1 g) and the mixture was hydrogenated in a Parr bom apparatus (500 psi H_2) for 7 hours. Boc-anhydride (2.95 g, 13.5 mmol) was then added followed by DIEA (6 ml, 66 mmol) and the reaction was stirred for 2 hours. The Pd/C catalyst was removed by filtration through Celite and the reaction mixture was partitioned between 500 ml of bicarbonate and 500 ml of dichloromethane and extracted

with dichloromethane (2 x 200 ml). The pH was then reduced to three with 10% citric acid and the mixture was extracted with dichloromethane (4 x 200 ml.) The combined acidic extracts were dried (sodium sulfate) and concentrated *in vacuo* to yield a brown oil. The crude mixture was purified by flash chromatography (10% MeOH/dichloromethane) to yield a white solid. (2.8 g, 69.5% yield). ^{1}H NMR (DMSO-d_6) δ 12.75 (br s, 1H), 9.15 (s, 1H), 7.14 (s, 1H), 6.64 (2, 1H), 4.90 (s, 2H), 3.68 (s, 3H), 1.46 (s, 9H).

Preparation of allyl 1-(carboxymethyl)-4-[[(*tert*-Butyloxy)carbonyl]amino]-2-carboxylate (52). To a solution of the Boc-methyl ester (**51**) (500 mg, 1.6 mmol) in 5 ml of dry allyl alcohol was added a solution of 60% sodium hydride (640 mg) dissolved in 10 ml of allyl alcohol. The gel-like mixture was refluxed for 30 minutes and cooled to room temperature. 100 ml of 10% citric acid was added and the reaction mixture was extracted with dichloromethane (3 x 200 ml). The combined organics were dried and concentrated *in vacuo* to yield the pure Boc allyl ester (**31**) as a brown oil. (408 mg, 82% yield). ^{1}H NMR (DMSO-d_6) δ 9.18 (s, 1H), 7.18 (s, 1H). 6.68 (s, 1H), 5.91- 6.01 (m, 1H), 5.21-5.34 (dd, 2H), 4.96 (s, 2H), 4.3 (d, 2H, J = 5.2 Hz), 1.45 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 170.4, 160.0, 152.9, 133.1, 123.4, 119.7, 117.8, 107.9, 78.8, 64.0, 50.0, 28.3; FABMS found 313.140, calc. 313.140.

Example 13. Preparation of *cyclo*-(-γ-ImPyImPy-γ-ImPyImPy(-G-Dp)-) (57)

Synthesis of the linear precursor H_2N-γ-ImPyImPy-γ-ImPyImPy(-G-Dp)-COOH (56). Boc-G-PAM resin (1.25 g, 0.25 mmol) was deprotected under standard conditions. Boc allyl monomer **52** (101 mg, 0.325 mmol) and HOBt (88 mg, 0.65 mmol) were dissolved in 400 μl DMF and DCC

(66 mg, 0.325 mmol) was added. After 15 minutes DCU was removed by filtration and the activated ester was added to the reaction vessel, followed by 1.5 ml DMF and 355 μl DIEA. The reaction was allowed to proceed for 12 hours providing Boc-Py(O-allyl)-G-resin (**53**). The remaining polyamide was synthesized by standard solid phase methods of this invention to provide H_2N-γ-ImPyImPy-γ-ImPyImPy(O-allyl)-G-resin (**54**). To remove the allyl protecting group, the resin was then treated with THF (2 x 200 ?? ml) and 10 ml of a solution of 593 μl n-butylamine and 250 μl formic acid in 25 ml THF was then added, followed by 280 mg $Pd_2(dba)_3$-$CHCl_3$ and 980 mg triphenylphosphine. The reaction mixture was shaken for 3 hours at room temperature, drained, rinsed with acetone (200 ml), 0.1M sodium *N,N*-diethyl-dithiocarbamate in water (50 ml, 2 x 1 min), acetone (200 ml), water (200 ml), 0.1M sodium *N,N*-diethyl-dithiocarbamate in water (50 ml, 2 x 1 min), acetone (200 ml), water (200 ml), methanol (200 ml), dichloromethane (200 ml), methanol (200 ml), and the resin was dried *in vacuo*. The polyamide was then cleaved and purified under standard conditions to yield the linear precursor (**56**) as a fluffy white solid. (105 mg, 45% yield). HPLC, r.t. 27.0 min.; ^{1}H NMR (DMSO-d_6) δ 10.37 (s, 1H), 10.29 (s, 1H), 10.23 (s, 1H), 10.22 (s, 1H), 10.18 (s, 1H), 9.96 (s, 1H), 9.92 (s, 1H), 9.86 (s, 1H), 9.31 (br s, 1H), 8.31 (t, 1H), 8.06 (t, 1H), 7.85 (t, 1H), 7.71 (br s, 3H), 7.53 (m, 2H), 7.44 (s, 1H), 7.36 (s, 1H), 7.189 (d, 1H, *J*=1.5 Hz), 7.12 (d, 1H, *J*=1.7 Hz), 6.97 (d, 1H, *J*=1.6 Hz), 6.94 (d, 1H, *J*=1.5 Hz), 4.97 (s, 2H), 3.96 (m, 6H), 3.94 (m, 9H), 3.85 (m, 6H), 3.79 (s, 3H), 3.66 (d, 1H, *J*=5.7 Hz), 3.23 (m, 4H), 2.98 (m, 2H), 2.79 (m, 2H), 2.71 (d, 6H, *J*=4.4 Hz), 2.33 (m, 4 Hz), 1.77 (m, 6H); MALDI-TOF MS. 1355.4, found 1355.7 calc.

Removal of the allyl group yielded no undesirable side products as determined by HPLC.

Synthesis of *cyclo*-(-γ-ImPyImPy-γ-ImPyImPy(-G-Dp)- (57). The linear precursor (**56**) (16 mg) was dissolved in 7 ml of DMF, DPPA (28 mg) was added followed by potassium carbonate (45 mg) and the reaction mixture rapidly shaken for 3 hours, upon which time the reaction was determined to be complete by HPLC analysis r.t. 29 min. The cyclic peptide was purified by preparatory HPLC. MALDI-TOF MS 1355.4 calc., 1355.7 found (Figure 19).

Example 14. Preparation of oligonucleotide-polyamide conjugates

Materials. The following additional materials are needed for synthesis of oligonucleotide-polyamide conjugates. 6-(4-monomethoxytritylamino)propyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite, 5'-amino-modifier C6. 12-(4-monomethoxytritylamino)propyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite, 5'-amino-modifier C12. dT CE (2-cyanoethyl) phosphoramidite, 0.45 M sublimed tetrazole in acetonitrile. THF/lutidine/Ac_2O (8:1:1), 10% MeIm in THF, 0.1M I_2 in THF/Pyridine/H_2O, 3% TCA/CH_2Cl_2, 5-methylcytidine CE (2-cyanoethyl) phosphoramidite, and bulk 500 Å dT-Icaa-CPG were purchased from Glen Research. All 10 μmole preparation columns were packed manually from bulk support. 1,2,3-benzotriazol-1-yl-4[[(tert-butyloxy)carbonyl]-amino]-1-methylpyrrole-2-carboxylate and 1,2,3-benzotriazol-1-yl-4[[(tert- butyloxy)carbonyl]-amino]-1-methylimidazole-2-carboxylate were prepared as previously described. (Baird and Dervan *Manuscript in Preparation*; Schnozler *et al.* (1992) Int. J. Pep. Prot. Res.

40:180-193; Grehn and Ragnarsson (1981 J. Org. Chem. 46:3492-3497; Grehn *et al.* (1990) Acta. Chem. Scand. 44:67-74.). 0.0002M potassium cyanide/pyridine, and acetic anhydride (Ac_2O) were purchased from Applied Biosystems. HPLC analysis was performed either on a HP 1090 M analytical HPLC or a Beckman Gold system using a RAINEN C18, Microsorb MV, 5 µm, 300 x 4.6 mm reversed phase column in 100 mM ammonium acetate, pH 4.9 with acetonitrile as eluent and a flow rate of 1.0 ml/min, gradient elution 1.0% acetonitrile/min. Oligonucleotide conjugates were purified by FPLC (Pharmacia) on a ProRPC HR 10/10 reversed phase column using a linear gradient from 0 to 40% acetonitrile in 55 minutes, 100 mM triethylammonium acetate, pH 7.0.

Preparation of ImPyPy-CONH$(CH_2)_6$-P(O)$_4$TTTTTT^{m}C^{m}CTTT-3' (62) (SEQ ID NO:7). The oligonucleotide DMT-TTTTTT^{m}C^{m}CTTT-CPG was prepared on an Applied Biosystems Model 394B DNA synthesizer using a manually prepared 10 µmole synthesis column and a standard 10 µmole synthesis cycle. C_6-Aminomodifier-MMT (100 µmole) was dissolved in 1,100 µl of anhydrous acetonitrile. vortexed vigorously, and placed on the synthesizer. The amino modifier was added by machine synthesis using a modified 10 µmole synthesis cycle with an extended 10 minute coupling time and the MMT group left on. The column was manually washed with 50 ml of 3% trichloroacetic acid/dichloromethane until a yellow color was no longer observed in the wash (approximately 12 minutes). The column was then washed with 15 ml dichloromethane and dried *in vacuo*. The CPG was transferred to a 5 ml glass manual peptide reaction vessel and washed with DMF (30 seconds). A sample was taken for ninhydrin test and an absorbance consistent with 50 µmole/gram substitution was found.

Boc-pyrrole-OBt ester (**9**) (70 mg, 200 μmole) was dissolved in DMF (600 μl) and DIEA (68 μl) was added. The coupling mixture was added to the reaction vessel and the mixture was shaken for 60 minutes. The resin was washed with DMF (30 seconds), dichloromethane (30 seconds) and 65% TFA/CH_2Cl_2/0.5M PhSH (30 seconds). The resin was shaken in 65% TFA/CH_2Cl_2/0.5M PhSH for 20 minutes, drained, washed with dichloromethane (30 seconds) followed by DMF (30 seconds). A second equivalent of Boc-pyrrole-OBt was added under identical conditions to the first, and the reaction shaken for 1 hour, washed with DMF (30 seconds), dichloromethane (30 seconds) and treated with 65% TFA/CH_2Cl_2/0.5M PhSH as described for the first deprotection. N-methylimidazole-2-carboxylic acid (133 mg) was activated in 1 ml of DMF with HOBt/DCC as described above and added to the reaction vessel with DIEA (200 μl) and the reaction was shaken to shake for 2 hours. The CPG was washed with DMF (30 seconds). dichloromethane (30 seconds) and dried *in vacuo*. The entire CPG (approximately 210 mg) was placed in 2 ml of 0.1M NaOH and heated at 55°C for 15 hours. The CPG was removed by filtration through a polypropylene filter, and 1 ml of 1M triethylammonium acetate, pH 7, was added and the reaction diluted to 10 ml total volume with water. The mixture was purified in two separate portions by FPLC. In each run the conjugate was triple injected in 1.5 ml volume portions. Collected fractions were lyophilized, and relyophilized from water to yield the desired conjugate ImPyPy-$CONH(CH_2)_6P(O)_4$-TTTTTT^{m}C^{m}CTTT-3' (**62**) (SEQ ID NO:7) (15 mg, 37% yield).

Characterization of ImPyPy-$CONH(CH_2)_6P(O)_4$-TTTTTT^{m}C^{m}CTTT-3' (**62**). Analytic HPLC (10 nmole). r.t., 14.8 min.;

UV(H_2O/DMSO)$\lambda_{max}(\epsilon)$, 260 (118,000), 304 (33,000); 18,800u. Enzymatic digestion (10 nmole), 7.8 min. (mC, 2441u), 9.0 min. (T, 15,235u) 26.2 min. (peptide, 4257u (260), 1843u 340); ^{1}H NMR (D_2O) δ 7.71 (s, 1H), 7.69 (s, 1H), 7.50 (m, 9H), 7.19 (s, 1H), 7.18 (s, 1H), 7.12 (d, 1H, J = 1.2 Hz), 7.05 (d, 1H, J = 1.3Hz), 6.89 (d, 1H, J = 1.3Hz), 6.67 (d, 1H, J = 1.3Hz), 6.11 (m, 11H), 4.89, 4.76, 4.70, 4.22, 4.01, 3.91, 3.88, 3.75, 3.42, 3.11, 2.40, 2.25, 2.20, 1.80, 1.75, 1.41, 1.23; MALDI-TOF MS, calc. M-1 3814.3, found 3813.5.

Synthesis of AcImPyPy-CONH$(CH_2)_{12}$P(O)$_4$-TTTTTT^{m}C^{m}CTTT-3' (**63**) (SEQ ID NO:8). Boc-PyPy-CONH$(CH_2)_{12}$P(O)$_4$-TTTTTT^{m}C^{m}CTTT-- CPG was assembled as described above for compound **62**. The N-boc group was removed with 65% TFA/CH_2Cl_2/0.5M PhSH and the resin was treated with a solution of Boc-Im-OBt (**13**) (70 mg), DIEA (68 µl) and DMF (600 µl), and shaken for 1 hour. The CPG was washed with DMF (30 seconds), dichloromethane (30 seconds) and dried *in vacuo*. One third of the CPG, 70 mg. was removed from the synthesis and placed in a 10 µmole DNA synthesis column. Two syringes were used simultaneously to manipulate reagents into and out of the column. The column was washed with dichloromethane and *carefully* treated with 65% TFA/CH_2Cl_2/0.5M PhSH to remove the N-Boc group. The column was carefully washed with dichloromethane (20 ml) and DMF (20 ml) and then treated with acetylation mixture for 1 hour, washed with DMF (20 ml) and CH_2Cl_2 (20 ml) and the cartridge dried *in vacuo*. The resin was removed from the column and placed in 1 ml 0.1M NaOH at 55°C for 15 hours. The CPG was removed by filtration, 1 ml of 1 M pH 7 triethylammonium acetate was added and the mixture purified by FPLC. The appropriate fractions were collected and concentrated *in vacuo* to give AcImPyPy-CONH$(CH_2)_{12}$P(O)$_4$-TTTTTT^{m}C^{m}CTTT-3' (**63**) (SEQ ID NO:8)

(336 nmole, 10% yield).

Characterization of AcImPyPy-CONH$(CH_2)_{12}P(O)_4$-TTTTTT^{m}C^{m}CTTT-3' (**63**). Analytic HPLC (10 nmole), r.t., 19.7 min.; UV(H_2O/DMSO)$\lambda_{max}(\epsilon)$, 260 (110,000), 304 (36,000); 13,000u, Enzymatic digestion (10 nmole), 7.7 min. (mC, 1551u), 8.9 min. (T, 11374u), 33.2 min. (peptide, 1646 u (260), 1281u 340); MALDI-TOF MS, calc. M-1 3953.5, found 3952.9.

Preparation of Boc-γ-ImPyPy-CONH$(CH_2)_{12}P(O)_4$-TTTTTT^{m}C^{m}CTTT-3' (**64**) (SEQ ID NO:9). A sample of Boc-ImPyPy-CONH$(CH_2)_{12}P(O)_4$-TTTTTT^{m}C^{m}CTTT--CPG (140 mg), prepared by the general procedures described above was placed in a 10 μmole DNA synthesis column. The column was washed with dichloromethane and *carefully* treated with 65% TFA/CH_2Cl_2/0.5M PhSH to remove the N-Boc group. The column was then carefully washed with dichloromethane (20 ml) and DMF (20 ml) and then treated with the HOBt ester of Boc-γ prepared *in situ* (1 mmol, 200 μl DIEA, 1 ml DMF) and allowed to react for 1 hour. The CPG was washed with DMF (20 ml), dichloromethane (20 ml), and dried *in vacuo*. The resin was removed from the column and placed in 1 ml 0.1M NaOH at 55°C for 15 hours. The CPG was removed by filtration, 1 ml of 1M pH 7 triethylammonium acetate was added and the mixture was purified by FPLC. The appropriate fractions were collected and concentrated *in vacuo* to give Boc-γ-ImPyPy-CONH$(CH_2)_{12}P(O)_4$-TTTTTT^{m}C^{m}CTTT-3' (**64**) (SEQ ID NO:9) (343 nmole, 5% yield).

Characterization of Boc-γ-ImPyPy-CONH$(CH_2)_{12}P(O)_4$-TTTTTT^{m}C^{m}CTTT-3' (**64**). UV(H_2O/DMSO)$\lambda_{max}(\epsilon)$ 260 (120,000), 304 (34,000); Analytic HPLC (5 nmole), r.t., 23.0 min., 6,000u, Enzymatic

digestion (5 nmole), 7.7 min. (mC, 779u), 8.9 min. (T, 5873 u) 42.1 min. (peptide, 767u (260), 597u 340) MALDI-TOF MS, calc. M-1 4094.7, found 4096.8.

Preparation of H_2N-γ-ImPyPy-$CONH(CH_2)_{12}P(O)_4$-$TTTTTT^mC^mCTTT$-3' (65) (SEQ ID NO:10). Boc-γ-ImPyPy-$CONH(CH_2)_{12}P(O)_4$-$TTTTTT^mC^mCTTT$-3' (64) (330 nmole) was treated with 400 µl of 65% TFA/CH_2Cl_2/0.5M PhSH for 30 minutes. 2 ml of 1M pH 7 triethylammonium acetate and 5 ml of water was added and the reaction mixture was vortexed, frozen, and lyophilized. The reaction mixture was then dissolved in 5 ml of 100 mM triethylammonium acetate and purified by FPLC. appropriate fractions were collected and concentrated *in vacuo* to give H_2N-γ-ImPyPy-$CONH(CH_2)_{12}P(O)_4$-$TTTTTT^mC^mCTTT$-3' (65) (240 nmole, 70% yield). MALDI-TOF MS, calc. M-1 3995.5, found 3999.7

Preparation of EDTA-γ-ImPyPy-$CONH(CH_2)_{12}P(O)_4$-$TTTTTT^mC^mCTTT$-3' (66) (SEQ ID NO:11). H_2N-γ-ImPyPy-$CONH(CH_2)_{12}P(O)_4$-$TTTTTT^mC^mCTTT$-3' (65) (SEQ ID NO:10) was dissolved in 500 µl 500 mM carbonate buffer (pH 9.5). 10 mg of the monoanhydride of EDTA was added and the reaction allowed to proceed for 15 minutes. After 15 minutes 1 ml of triethylammonium acetate, pH 7.0 was added with 4 ml of water and the reaction immediately purified by FPLC. The appropriate fractions were collected and concentrated *in vacuo* to give EDTA-γ-ImPyPy-$CONH(CH_2)_{12}P(O)_4$-$TTTTTT^mC^mCTTT$-3' (66) (70 nmole, 41% yield).

Example 15. Automated Synthesis of Polyamides

The manual solid phase method for synthesis of pyrrole and imidazole polyamides was adapted for use on an ABI 430A peptide synthesizer. Machine-assisted synthesis was performed on a 0.18 mmol scale (900 mg resin at 0.2 mmol/gram). Each cycle of amino acid addition involved: deprotection with approximately 80% TFA and 0.4 M thiophenol in dichloromethane for 3 minutes, draining the reaction vessel, and then deprotection for 17 minutes; 2 dichloromethane flow washes; an NMP flow wash; draining the reaction vessel; coupling for 1 hour with *in situ* neutralization, addition of DMSO/NMP, coupling for 30 minutes, addition of DIEA, coupling for 30 minutes; draining the reaction vessel; washing with dichloromethane, taking a resin sample for evaluation of the progress of the synthesis by HPLC analysis; capping with acetic anhydride/DIEA in dichloromethane for 6 minutes; and washing with dichloromethane.

The synthesizer was left in the standard hardware configuration for NMP-HOBt protocols. Reagent positions 1 and 7 (Figure 21) were DIEA, reagent position 2 was TFA/0.5M thiophenol, reagent position 3 was 70% ethanolamine/methanol, reagent position 4 was acetic anhydride, reagent position 5 was DMSO/NMP, reagent position 6 was methanol and reagent position 8 was 0.48 M HBTU. All pyrrole and imidazole monomers were preactivated, predissolved and filtered through an ISOLAB filter (cat. #QS-Q) before placing in a synthesis cartridge. Boc-Py-OBt ester (357 mg, 1 mmol) was dissolved in 2 ml of DMF and filtered into a synthesis cartridge. Boc-imidazole monomer (125 mg, 0.5 mmol) and HOBT (135 mg, 1.0 mmol) were dissolved in 500 μl of DMF, DCC (102 mg, 0.5 mmol) was added, and the mixture allowed to stand for 15 minutes. DMF (1.5 ml) was then added, DCU removed by filtration and the activated monomer placed in a synthesis

cartridge. Boc-γ-Im-COOH or Boc-PyIm-COOH (100 mg, approximately 0.3 mmol) and HBTU (118 mg, 0.3 mmol) were dissolved in 500 μl DMF, 100 μl DIEA was added and the solution allowed to stand for 3 minutes. 1.5 ml of DMF was added and the solution filtered into a synthesis cartridge. For capping with imidazole-2-carboxylic acid, no activation conditions were compatible with the delivery-line filters. Im-COOH (800 mg, approximately 6 mmol) and HBTU (1 g, 3 mmol) were combined in 2.5 ml DMF, 1 ml DIEA was added and the mixture allowed to stand for 15 minutes. At the initiation of the coupling cycle, the synthesis was interrupted, the reaction vessel vented with toggle switches 0 and 2, the activated monomer filtered through a 0.2μ nylon filter and added directly to the reaction vessel via syringe. Temporary attachment of the resin sampling tube to a syringe, provides an easy method for direct manual addition of regents to the reaction vessel. For coupling pyrrole to imidazole, Boc-Py-COOH (514 mg, 2 mmol) was dissolved in 2 ml dichloromethane, DCC (420 mg, 2 mmol) was added. and the solution allowed to stand for 10 minutes. DMAP (101 mg, 1 mmol) was then added and the solution allowed to stand for an additional 1 minute. The solution was filtered and manually added to the reaction vessel at the initiation of coupling via syringe. For both coupling procedures where manual addition was necessary, the standard pyrrole-imidazole polyamide activator cycle was used in conjunction with an empty synthesis cartridge. Aliphatic amino acids (2 mmol) in 2 ml DMF were activated with HBTU (718 mg, 1.9 mmol), filtered and placed in a synthesis cartridge. Alternatively, the amino acid (1.5 mmol) was placed dry in a cartridge and 0.48 M HBTU (3 ml, 1.4 mmol) added using a calibrated delivery loop from reagent bottle eight, followed by the addition of 1 ml DIEA from reagent bottle 7 using a calibrated delivery loop, 3 minute

mixing of the cartridge, direct transfer to the concentrator without rinse, and subsequent transfer to the reaction vessel without rinse.

Preparation of ImPyPy-β-PyPyPy-G-Dp. The Polyamide ImPyPy-β-PyPyPy-G-Dp was prepared by the general automated solid phase methods described above to yield a white powder. (17.2 mg, 57% recovery). HPLC, r.t. 26.5; UV(H_2O/DMSO)λmax (ε), 246 (46,500), 312 (54,800); ^1H NMR (DMSO-d_6) δ 10.54 (s, 1H), 9.92 (s, 1H), 9.90 (m, 3H), 9.23 (br s, 1H), 8.27 (t, 1H, *J*=5.5 Hz), 8.06 (t, 1H, *J*=6.3 Hz), 8.03 (t, 1H, *J*=6.2 Hz), 7.39 (s, 1H), 7.26 (d, 1H, *J*=1.7 Hz), 7.20 (m, 2H), 7.17 (m, 2H), 7.13 (m, 2H), 7.04 (d, 1H), *J*=1.5 Hz), 6.87 (d, 1H, *J*=1.8 Hz), 6.83 (d, 1H, *J*=1.8 Hz), 3.97 (s, 3H), 3.82 (m, 15H), 3.78 (d, 2H, *J*=3.4 Hz), 3.27 (m, 4H), 3.15 (m, 2H), 3,79 (m, 2H), 2,76 (d, 6H, *J*=4.9 Hz), 1.78 (quintet, 2H, *J*=6.6 Hz); MALDI-TOF MS 950.2; FABMS *m/e* 949.458 (M^+H^+ 949.455, calc. for $C_{45}H_{57}N_{16}O_8$).

Table 1. Polyamides synthesized by the solid phase method of this invention.

ImPyPy-G-PyPyPy-G-Dp
AcImPyPy-γ-PyPyPy-G-Ta
AcImPyPy-γ-PyPyPy-G-Ta-EDTA
AcPyPyPy-γ-ImImPy-G-Dp
ImPyPy-γ-PyPyPy-ß-Dp
ImPyPy-ß-PyPyPy-γ-ImPyPy-ß-PyPyPy-ß-Dp
AcImPyPy-γ-PyPyPy-ß-Dp
H_2N-γ-ImPyPy-ß-PyPyPy-G-Dp
HOOC-Suc-ImPyPy-γ-PyPyPy-G-Dp
AcPyImPy-G-Dp
H_2N-PyPyPy-G-Dp
ImPyPy-Dala-PyPyPy-G-Dp
ImPyPy-γ-PyPyPy-G-Dp
ImPyPy-Lala-PyPyPy-G-Dp
ImPyPy-AIB-PyPyPy-G-Dp
ImPyImPy-ß-Dp
ImPyPy-ß-PyPyPy-G-Dp
ImImPy-γ-PyPyPy-ß-Dp
AcImPyPy-G-PyPyPy-G-Dp
ImPyPy-G-PyPyPy-ß-Dp
ImPyPy-ß-PyPyPy-γ-ImPyPy-ß-PyPyPy-ß-Ta
ImPyPy-γ-ImPyPy-ß-PyPyPy-G-Dp
ImPyPy-ß-PyPyPy-G-Ta
ImPyPy-G-PyPyPy-G-Ta
AcPyPyPyPyPyPy-G-Ta
AcImPyPy-γ-PyPyPy-Dp
ImPyPyPyPyPyPy-G-Ta-EDTA
ImPyPy-Lglu-PyPyPy-G-Dp
ImPyPyPyPyPyPy-G-Dp
ImPyPyPyPyPyPy-G-Ed
AcImPyPy-G-PyPyPy-G-Ta-EDTA
AcImImPy-γ-PyPyPy-G-Ta
AcPyPyPy-γ-ImImPy-G-Ta
AcPyPyPy-γ-ImImPy-ß-Dp
AcPyPyPy-γ-ImImPy-G-Dp

Table 1. (Continued)

H_2N-ImPyPy-G-PyPyPy-G-Dp
EDTA-γ-ImPyPy-ß-PyPyPy-G-Dp
ImPyPy-γ-ImPyPy-G-PyPyPy-G-Dp
H_2N-γ-ImPyPy-ß-PyPyPy-G-Ta
AcImImPy-γ-PyPyPy-G-Dp
AcImPyPy-γ-PyPyPy-G-Dp
ImPy-G-Py-γ-ImPy-G-Py-ß-Dp
ImImPy-γ-ImPyPy-ß-PyPyPy-G-Dp
ImPyImPy-γ-ImPyImPy-ß-Dp
ImPyImPy-γ-PyPyPyPy-ß-Dp
ImImPyPy-γ-PyPyPyPy-ß-Dp
ImPyPy-ß-PyPyPy-G-Ta-EDTA
ImPyPy-G-PyPyPy-G-Ta-EDTA
AcImImPy-γ-PyPyPy-ß-Dp
AcImPyPy-G-PyPyPy-G-Ta
ImPyPy-G-PyPyPy-G-Ed
ImPyPy-γ-ImPyPy-ß-Dp
AcImPyPyPyPyPyPy-G-Ta-EDTA
AcPyPyPy-γ-ImImPy-G-Ta-EDTA
ImPyPy-transcyclopropyl-PyPyPy-ß-Dp
AcImPyPy-G-PyPyPy-G-Ta
PyPyPy-γ-ImImPy-G-Dp
ImImIm-ß-PyPyPy-ß-Dp
AcPyPyImPy-γ-PyPyPyPy-ß-Dp
AcImImPy-γ-PyPyPy-G-Dp
H_2N-ß-PyPyPy-γ-ImImPy-ß-ß-ß-ß-PyPyPy-γ-ImImPy-ß-Dp
(automated synthesis)
ImPyPyPy-γ-ImPyPyPy-ß-Dp
PyPyPy-γ-ImImPy-ß-Dp
PyPyPy-γ-ImImPy-G-Dp
DM-γ-ImPyPy-ß-PyPyPy-ß-Dp
ImPyPy-ß-ImImPyPy-γ-ImImPyPy-ß-Dp
ImPyPy-ß-PyPyPy-ß-Dp
ImImPyPy-γ-ImImPyPy-ß-Dp
ImPyPy-γ-ß-ß-ß-ß-Dp

Table 1. (Continued)

ImPyPy-γ-ß-PyPy-ß-Dp
ImPyPy-γ-ImPyPy-ß-PyPyPy-G-Ta-EDTA
H_2N-γ-ImPyPyPy-γ-PyPyPyPy(G-Dp)-COOH
ImImImIm-γ-PyPyPyPy-ß-Dp
ImPyPyPy-ß-ImImPyPy-γ-ImImPyPy-ß-Ta-EDTA
ImPyPyPy-γ-PyPyPyPy-ß-Dp
H_2N-ϵ-ImPyPy-G-PyPyPy-G-Dp
DMγ-ImPyPy-γ-ImPyPy-ß-ED
ImPyPyPy-γ-PyPyPyPy-Ta
ImPyPyPy-γ-PyPyPyPy-Ta-EDTA
ImPyPyPy-γ-ImPyPyPy-ß-Ta
ImPyPyPy-γ-ImPyPyPy-ß-Ta-EDTA
ImPyPyPy-γ-ImImImPy-ß-Ta
ImPyPyPy-γ-ImImImPy-ß-PyPyPyPy-ß-Ta
ImPyPy-Dala-PyPyPy-ß-Dp
ImPyPy-Lala-PyPyPy-ß-Dp
ImPyPy-ß-PyPyPy-Dala-Dp
ImPyPy-ß-PyPyPy-Lala-Dp
ImPyPy-γ-^{m}PyPyPy-ß-Dp
ImPy^{m}Py-γ-PyPyPy-ß-Dp
ImPyPy-ß-Py^{m}PyPy-ß-Dp
Im^{m}PyPy-ß-PyPyPy-ß-Dp
EDTA-γ-ImPyPy-G-PyPyPy-G-Dp
EDTA-γ-ImPyPy-G-PyPyPy-G-Ta-EDTA
EDTA-γ-ImPyPy-ß-PyPyPy-G-Ta-EDTA

* All compounds listed have be characterized by HPLC, ^{1}HNMR, MALDI-TOF mass spectroscopy and in some cases ^{13}C NMR.

Abbreviations.

Im = Imidazole	EDTA-ethylenediaminetetraacetic acid
Py = Pyrrole	ED = ethylenediamine
G = Glycine	Ta = 3,3-diamino-N-methylpropylamine
Dp = Dimethylaminopropylamine	Lala = L-alanine
Ac = Acetyl	ß = ß - alanine

Table 1. (Continued)

γ = γ-aminobutyric acid	Suc = Succinic acid
Dala = D-alanine	Lglu = L-Glutamic acid
AIB = alpha-isobutyric acid	ε = ε-aminohexanoic acid

DM-γ = N, N-dimethyl-γ-aminobutyric acid

Table 2. Standard protocol for manual synthesis of minor groove binding polyamides.

SYNTHESIS CYCLE	REAGENTS	TIME/MODE
1) Deprotect	65% TFA/CH_2Cl_2/PhSH	1 x 30 s flow 1 x 1 min shake 1 x 30 s flow 1 x 20 min shake
2) Wash	CH_2Cl_2 DMF	1 x 1 min flow 1 x 30 s flow 1 x 1 min shake
(take sample for picric acid test)		
3) Couple	HOBt acid, DIEA	45 min shake
(take sample for picric acid test)		
4) Wash	DMF CH_2Cl_2	1 x 30 s flow 1 x 30 s flow

Table 3. Apparent First-Order Association Constants (M^{-1}) for selected polyamides.[a]

Binding Site	Polyamide			
	ImPyPy-G-PyPyPy-Dp	ImPyPy-G-PyPyPy-G-Dp	ImPyPy-G-PyPyPy-β-Dp	SEQ ID NO:
5'-AAAAAGACAAAAA-3'	1.1 (±0.1) x 10^8	7.0 (±1.2) x 10^7	1.0 (±0.2) x 10^8	2
5'-ATATAGACATATA-3'	6.6 (±0.4) x 10^6	≈3.5 (±1.4) x 10^6	1.0 (±0.2) x 10^7	3
5'-TGTTAAACA-3'	1.4 (±0.1) x 10^8	≈1.7 (±0.7) x 10^6	3.4 (±0.5) x 10^7	4
5'-TGTAAAACG-3	5.5 (±0.3) x 10^7	≈3.0 (±1.5) x 10^6	4.0 (±1.3) x 10^7	?
5'-TGTGCTGCAAG-3'	5.4 (±0.2) x 10^7	<1 x 10^6	3.7 (±1.0) x 10^7	6

[a] Values reported are the mean values obtained from three DNase I footprint titration experiments. The standard deviation for each value is indicated in parentheses.

Table 4.* Illustrative Cyclic Polyamides.

cyclo-(ImPyImPy-γ-ImPyImPy-(G-Dp)-γ-)

cyclo(ImPyImPy-γ-ImPyImPy(G-Dp))

cyclo(ImPyPyPy-γ-PyPyPyPy(G-Dp))

H_2N-γ-ImPyImPy-γ-ImPyImPy(G-Dp)-COOH

H_2N-γ-ImPyImPy-γ-ImPyImPy(G-Dp)-COOH

* Abbreviations.

Im = Imidazole
Py = Pyrrole
G = Glycine
Dp = Dimethylaminopropylamine
γ = γ-aminobutyric acid

Table 5. Oligonucleotide-polyamide conjugates.

OLIGONUCLEOTIDE-POLYAMIDE CONJUGATE*	SEQ ID NO:
ImPyPy-C_6-P(O)$_4$TTTTTT^{m}C^{m}CTTT-3'	7
AcImPyPy-C_{12}-P(O)$_4$TTTTTT^{m}C^{m}CTTT-3'	8
Boc-γ-ImPyPy-C_{12}-P(O)$_4$TTTTTT^{m}C^{m}CTTT-3'	9
H_2N-γ-ImPyPy-C_{12}-P(O)$_4$TTTTTT^{m}C^{m}CTTT-3'	10
EDTA-γ-ImPyPy-C_{12}-P(O)$_4$TTTTTT^{m}C^{m}CTTT-3'	11
ImPyPy-C_6-P(O)$_4$TTT^{m}C^{m}CTTTTTT-3'	12
AcImPyPy-C_{12}-P(O)$_4$-TTT^{m}C^{m}CTTTTTT-3'	13
H_2N-γ-ImPyPy-C_5-P(O)$_4$-TTT^{m}C^{m}CTTTTTT-3'	14
H_2N-γ-ImPyPy-C_8-P(O)$_4$-TTT^{m}C^{m}CTTTTTT-3'	15
H_2N-γ-ImPyPy-C_{10}-P(O)$_4$-TTT^{m}C^{m}CTTTTTT-3'	16
H_2N-γ-ImPyPy-C_{12}-P(O)$_4$-TTT^{m}C^{m}CTTTTTT-3'	17
H_2N-γ-ImPyPy-C_6-P(O)$_4$(CH_2)$_8$(NH)TTT^{m}C^{m}CTTTTTT-3'	18
Dp-G-PyPyPy-G-PyPyIm-ε-DSA-(NH)TTT^{m}C^{m}CTTTTTT-3'	19
Dp-G-PyPyPy-G-PyPyIm-ε-DSG-(NH)TTT^{m}C^{m}CTTTTTT-3'	20
Dp-G-PyPyPy-G-PyPyIm-ε-DSS-(NH)TTT^{m}C^{m}CTTTTTT-3'	21

Dm-γ-ImPyPy-γ-ImPyPy-ß-ED-DSG-(NH)TTT^{m}C^{m}CTTTTTT-3'	22
DMγ-ImPyPy-γ-ImPyPy-ß-ED-DSA-(NH)TTTmCmCTTTTTT-3'	23

* Abbreviations.

C_6 represents $CONH(CH_2)_6$
C_{12} represents $CONH(CH_2)_{12}$
^{m}C = 5-methylcytidine
ETDA = ethylenediaminetetraacetic acid
γ = γ-aminobutyric acid
DSA = Adipic acid
DSG = Glutaric acid
DSS = Suberic acid
(NH)T = 2',5'-dideoxy-5'-aminothymidine
Im = Imidazole
Py = Pyrrole
G = Glycine
Dp = Dimethylaminopropylamine
ϵ = ϵ-aminohexanoic acid
β = β-alanine
ED = ethylenediamine
$C_8 = CONH(CH_2)_8$
$C_{10} = CONH(CH_2)_{10}$

WHAT IS CLAIMED IS:

1. A method for preparing polyamides containing imidazole and pyrrole carboxamides on a solid support comprising:

(a) preparing a resin for attachment of the polyamide by reacting the resin with a resin linkage agent;

(b) protecting and activating an amino acid, containing an amino group and a carboxyl group;

(c) sequentially adding the protected and activated amino acids to the solid support beginning with the carboxy terminal amino acid, thereby forming the desired polyamide;

(d) deprotecting the amino acids of the polyamide;

(e) cleaving the polyamide from the resin; and

(f) purifying the polyamide.

2. The method of claim 1 further comprising:

(g) reacting the polyamide with EDTA prior to purification.

3. The method of claim 1 wherein the resin is a polystyrene resin.

4. The method of claim 1 wherein resin linkage agent is selected from the group consisting of tert-butyloxycarbonylaminoacyl-pyrrole-4-(oxymethyl)phenylacetic acid (Boc-Py-PAM), tert-butyloxycarbonylaminoacyl-pyrrole 4-(oxymethyl)benzoic acid (Boc-Py-BAM), tert-butyloxycarbonylaminoacyl-imidazole 4-(oxymethyl)phenylacetic

acid (Boc-Im-PAM), tert-butyloxycarbonylaminoacyl-imidazole 4-(oxymethyl)benzoic acid (Boc-Im-BAM).

5. The method of claim 1 wherein the resin is prepared with a low substitution ratio of a spacer molecule prior to attachment of a resin linkage agent.

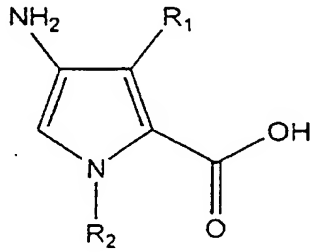
6. The method of claim 5 wherein the spacer is selected from the group consisting of glycine, β-alanine (β), glycine-PAM, or glycine-BAM.

7. The method of claim 6 wherein the substitution ratio is 0.2 to 0.3 mmol/gram.

8. The method of claim 1 wherein said amino acid is selected from the group consisting of an amino acid monomer or an amino acid dimer.

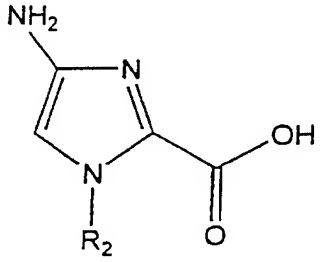
9. The method of claim 8 wherein the amino acid monomer is selected from the group consisting of a pyrrole amino acid, an imidazole amino acid, an aromatic amino acid or an aliphatic amino acid.

10. The method of claim 9 wherein said pyrrole amino acid has the following structure:



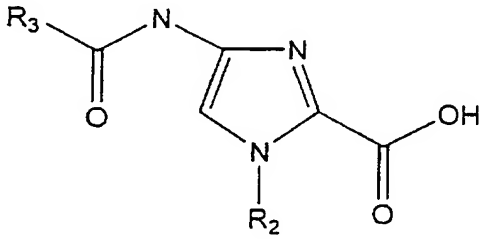
wherein R_1 is selected from the group consisting of H, CH_3, OH, NH_2, Cl or CF_3; R_2 is selected from the group consisting of a C1-C10 alkyl group, such as methyl, ethyl, or isopropyl, a C1-C10 alkene, or a C1-C10 alkyne, such as -$CCCH_3$.

11. The method of claim 9 wherein said imidazole amino acid has the following structure:



wherein R_2 is selected from the group consisting of a C1-C10 alkyl group, such as methyl, ethyl, or isopropyl, a C1-C10 alkene, or a C1-C10 alkyne, such as -$CCCH_3$.

12. The method of claim 8 wherein said amino acid dimer has the following structure:



wherein R_2 is selected from the group consisting of a C1-C10 alkyl group, such as methyl, ethyl, or isopropyl, a C1-C10 alkene, or a C1-C10 alkyne, such as -$CCCH_3$ and R_3 is an amino acid selected from the group consisting of a pyrrole

amino acid, an imidazole amino acid, an aromatic amino acid or an aliphatic amino acid or any chemical modifications thereof.

13. The method of claim 1 wherein the amino group of the amino acid is protected with a protecting group selected from the group consisting of tert-butoxy carbonyl (Boc-) or 9-fluoroenylmethylcarbonyl (Fmoc-).

14. The method of claim 1 wherein the carboxyl group of the amino acid is activated by the formation of the oxybenzotriazole (-OBt) ester.

15. The method of claim 1 wherein the carboxyl group of the amino acid is activated by the formation of the symmetric anhydride.

16. The method of claim 1 wherein the amino acids of the polyamide are deprotected by reaction with trifluoroacetic acid in the presence of a cation scavenger.

17. The method of claim 16 wherein the cation scavenger is selected from the group consisting of thiophenol, methyl ethyl sulfide or ethanedithiol.

18. The method of claim 1 wherein the polyamide is cleaved from the resin with $Pd(OAc)_2$.

19. The method of claim 1 wherein the polyamide is cleaved from the resin by amminolysis with an amine selected from the group consisting of dimethylaminopropylamine (Dp), 3,3'-diamino-N-methyldipropylamine (Ta), or ethylene- diamine (ED), or β-alanine(β).

20. An imidazole and pyrrole carboxamide polyamide produced according to the method of claim 1.

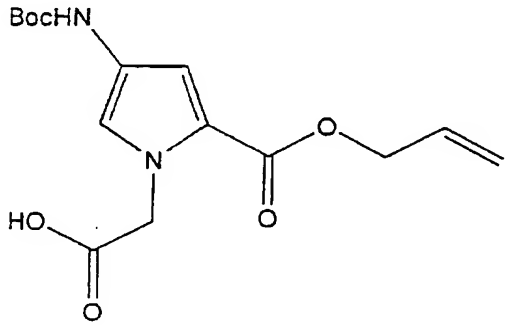
21. The polyamide of claim 20 wherein said polyamide is selected from the group of polyamides set forth in Table 1.

22. Polyamides able to bind to the minor-groove of double-stranded DNA produced by the method of claim 1.

23. A method for preparing cyclic polyamides containing imidazole and pyrrole carboxamides on a solid support comprising:

(a) preparing a resin for attachment of the polyamide by reacting the resin with a resin linkage agent;

(b) reacting the resin with a protected allyl ester pyrrole monomer having the following structure:



(c) protecting and activating an amino acid, containing an amino group and a carboxyl group;

(d) sequentially adding the protected and activated amino acids to the solid support, thereby forming the desired polyamide;

(d) removing the allyl ester with a Pd catalyst;

(e) cleaving the polyamide from the resin; and

(f) cyclizing and purifying the cyclized polyamide.

24. An cyclic imidazole and pyrrole carboxamide polyamide produced according to the method of claim 23.

25. The polyamide of claim 24 wherein said polyamide is selected from the group of polyamides set forth in Table 4.

26. Cyclic polyamides able to bind to the minor-groove of double-stranded DNA produced by the method of claim 23.

27. A method for preparing polyamide-oligonucleotide conjugates containing imidazole and pyrrole carboxamides comprising:

(a) preparing an oligonucleotide on a solid support;

(b) protecting and activating an amino acid;

(c) sequentially adding the protected and activated amino acids to the oligonucleotide beginning with the carboxy terminal amino acid. thereby forming the desired polyamide-oligonucleotide conjugate;

(d) deprotecting the amino acid of the polyamide-oligonucleotide conjugate;

(e) cleaving the polyamide-oligonucleotide conjugate from the solid support; and

(f) purifying the polyamide-oligonucleotide.

28. The method of claim 27 further comprising:

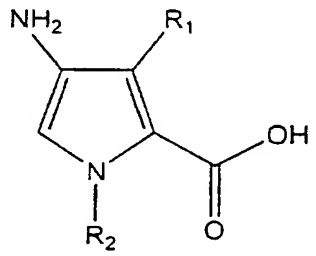
(g) reacting the polyamide-oligonucleotide with EDTA prior to purification.

29. The method of claim 27 wherein the solid support is porous glass.

30. The method of claim 27 wherein said amino acid is selected from the group consisting of an amino acid monomer or an amino acid dimer.

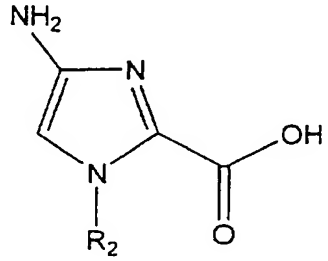
31. The method of claim 30 wherein the amino acid monomer is selected from the group consisting of a pyrrole amino acid, an imidazole amino acid, an aromatic amino acid or an aliphatic amino acid.

32. The method of claim 31 wherein said pyrrole amino acid has the following structure:



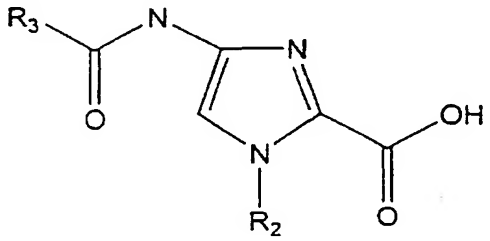
wherein R_1 is selected from the group consisting of H, CH_3, OH, NH_2, Cl or CF_3; R_2 is selected from the group consisting of a C1-C10 alkyl group, such as methyl, ethyl, or isopropyl, a C1-C10 alkene, a C1-C10 alkyne, such as -$CCCH_3$.

33. The method of claim 31 wherein said imidazole amino acid has the following structure:



wherein R_2 is selected from the group consisting of a C1-C10 alkyl group, such as methyl, ethyl, or isopropyl, a C1-C10 alkene, or a C1-C10 alkyne, such as -$CCCH_3$.

34. The method of claim 30 wherein said amino acid dimer has the following structure:



wherein R_2 is selected from the group consisting of a C1-C10 alkyl group, such as methyl, ethyl, or isopropyl, a C1-C10 alkene, or a C1-C10 alkyne, such as -$CCCH_3$ and R_3 is an amino acid selected from the group consisting of pyrrole

amino acid, an imidazole amino acid, an aromatic amino acid or an aliphatic amino acid or any chemical modification thereof.

35. The method of claim 27 wherein the amino group of the amino acid is protected with a protecting group selected from the group consisting of tert-butoxy carbonyl (Boc-), 9-fluoroenylmethylcarbonyl (Fmoc-).

36. The method of claim 27 wherein the amino acid is activated by the formation of the oxybenzotriazole (-OBt) ester.

37. The method of claim 27 wherein the amino acid is activated by the formation of the symmetric anhydrides.

38. The method of claim 27 wherein the amino acids of the polyamide-oligonucleotide conjugate are deprotected by reaction with trifluoroacetic acid in the presence of thiophenol.

39. The method of claim 27 wherein the polyamide-oligonucleotide is cleaved from the support with sodium hydroxide.

40. An imidazole and pyrrole carboxamide polyamide-oligonucleotide conjugate produced according to the method of claim 27.

41. The polyamide-oligonucleotide conjugate of claim 40 wherein said polyamide is selected from the group of polyamides set forth in Table 5.

42. Polyamide-oligonucleotide conjugates able to bind to the minor-groove of double-stranded DNA produced by the method of claim 27.

43. A method for preparing polyamide-protein conjugates containing imidazole and pyrrole carboxamides comprising:

(a) preparing an protein on a solid support;

(b) protecting and activating an amino acid;

(c) sequentially adding the protected and activated amino acids to the protein beginning with the carboxy terminal amino acid, thereby forming the desired polyamide-protein conjugate;

(d) deprotecting the amino acids of the polyamide-protein conjugate;

(e) cleaving the polyamide-protein conjugate from the solid support; and

(f) purifying the polyamide-protein conjugate.

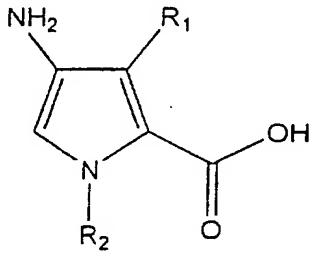
44. The method of claim 43 further comprising:

(g) reacting the polyamide-protein conjugate with EDTA prior to purification.

45. The method of claim 43 wherein said amino acid is selected from the group consisting of an amino acid monomer or an amino acid dimer.

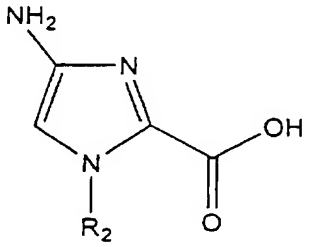
46. The method of claim 45 wherein the amino acid monomer is selected from the group consisting of a pyrrole amino acid, an imidazole amino acid, an aromatic amino acid or an aliphatic amino acid.

47. The method of claim 46 wherein said pyrrole amino acid has the following structure:



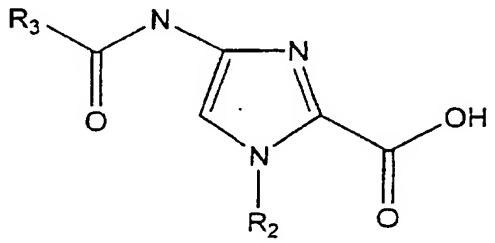
wherein R_1 is selected from the group consisting of H, CH_3, OH, NH_2, Cl or CF_3; R_2 is selected from the group consisting of a C1-C10 alkyl group, such as methyl, ethyl, or isopropyl, a C1-C10 alkene, a C1-C10 alkyne, such as -$CCCH_3$.

48. The method of claim 46 wherein said imidazole amino acid has the following structure:



wherein R_2 is selected from the group consisting of a C1-C10 alkyl group, such as methyl, ethyl, or isopropyl, a C1-C10 alkene, a C1-C10 alkyne, such as -$CCCH_3$.

49. The method of claim 45 wherein said amino acid dimer has the following structure:



wherein R_2 is selected from the group consisting of a C1-C10 alkyl group, such as methyl, ethyl, or isopropyl, a C1-C10 alkene, a C1-C10 alkyne, such as -$CCCH_3$ and R_3 is an amino acid selected from the group consisting of pyrrole amino acid, an imidazole amino acid, an aromatic amino acid or an aliphatic amino acid or any chemical modification thereof.

50. The method of claim 43 wherein the amino group of the amino acid is protected with a protecting group selected from the group consisting of tert-butoxy carbonyl (Boc-) or 9-fluoroenylmethylcarbonyl (Fmoc-).

51. The method of claim 43 wherein the amino acid is activated by the formation of the oxybenzotriazole (-OBt) ester.

52. The method of claim 43 wherein the amino acid is activated by the formation of the symmetric anhydrides.

53. An imidazole and pyrrole polyamide-protein conjugate produced according to the method of claim 43.

54. Polyamide-protein conjugates able to bind to the minor-groove of double-stranded DNA produced by the method of claim 43.

55. A computer readable storage medium, when inserted to a peptide synthesizer performs the following steps:

(a) transferring a predissolved pyrrole to a concentrator center;

(b) adding DIEA and an aeration to the concentrator center;

(c) treating a resin in a reaction center with TFA/PhSH;

(d) draining the TFA/PhSH from the reaction center;

(e) transferring the contents of the concentrator center to the reaction center;

(f) starting to shake the reaction center;

(g) adding DMSO to the reaction center;

(h) adding DIEA to the reaction center;

(i) draining the reaction center;

(j) adding Ac_2O to the reaction center;

(k) draining the reaction center; and

(l) stopping the shaking after two hours have lapsed.

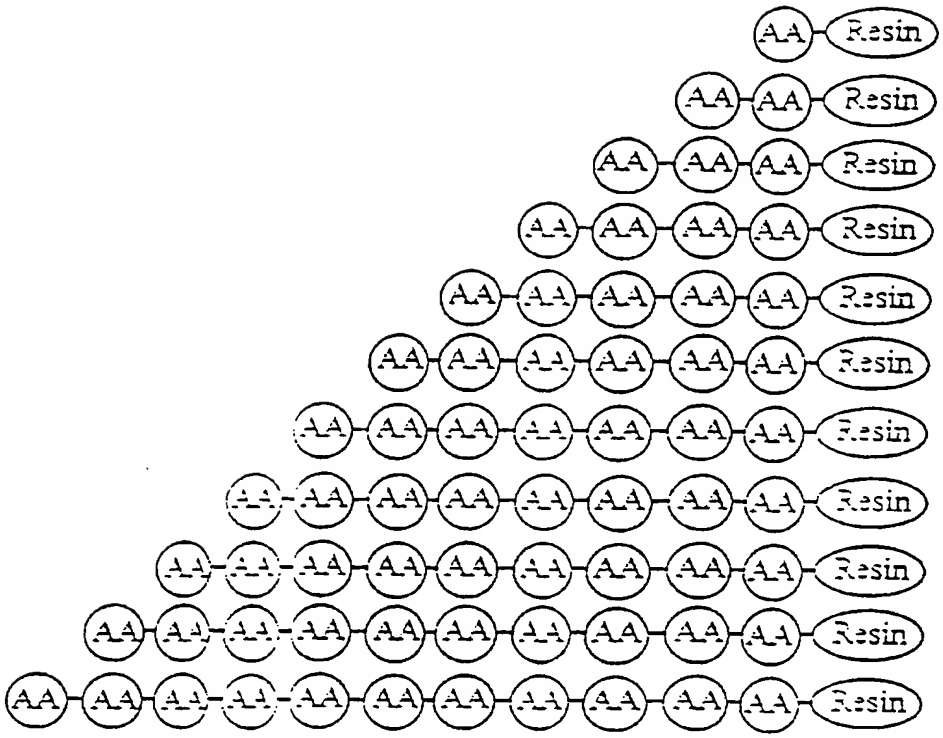

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Fig. 1

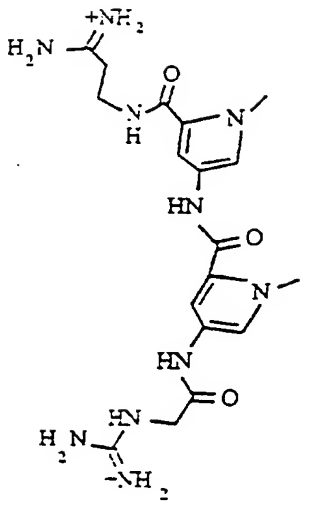


Fig. 2A

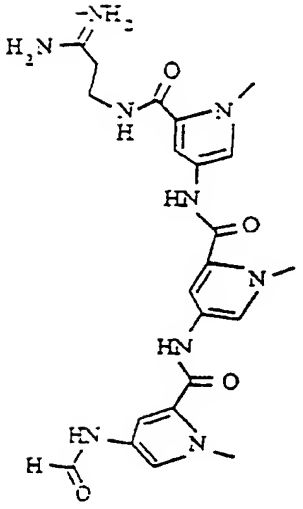


Fig. 2B

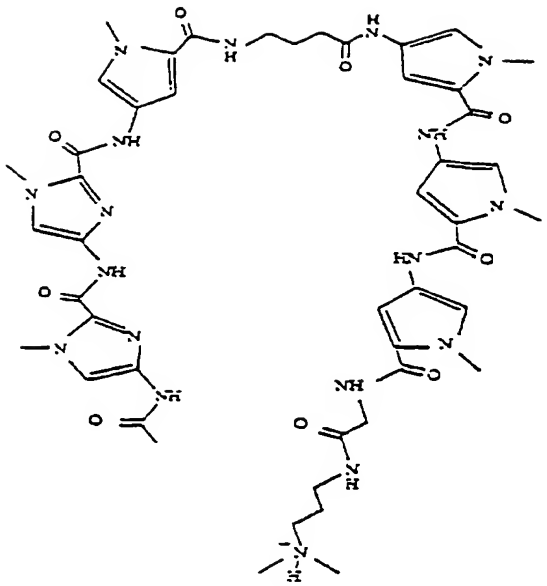


Fig. 3A

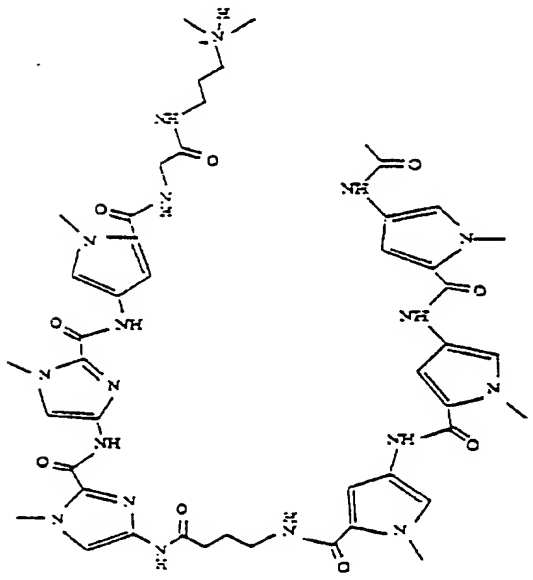


Fig. 3B

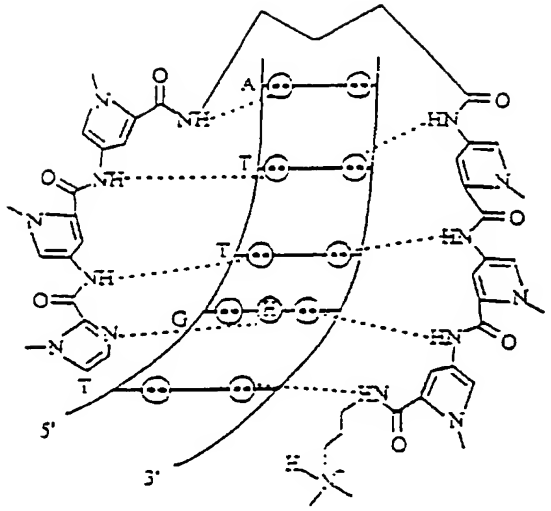


Fig. 4A

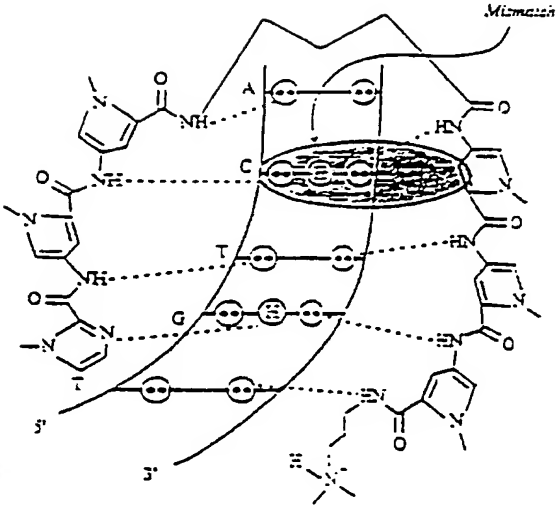


Fig. 4B

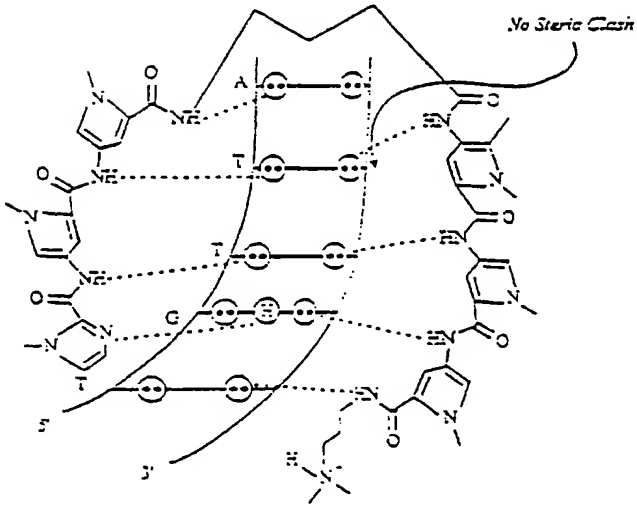


Fig. 4C

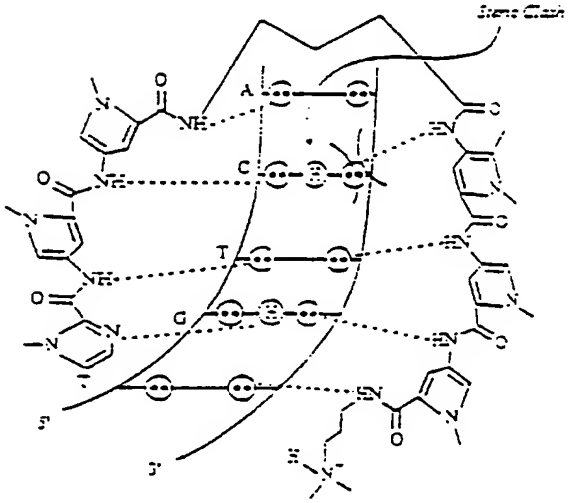


Fig. 4D

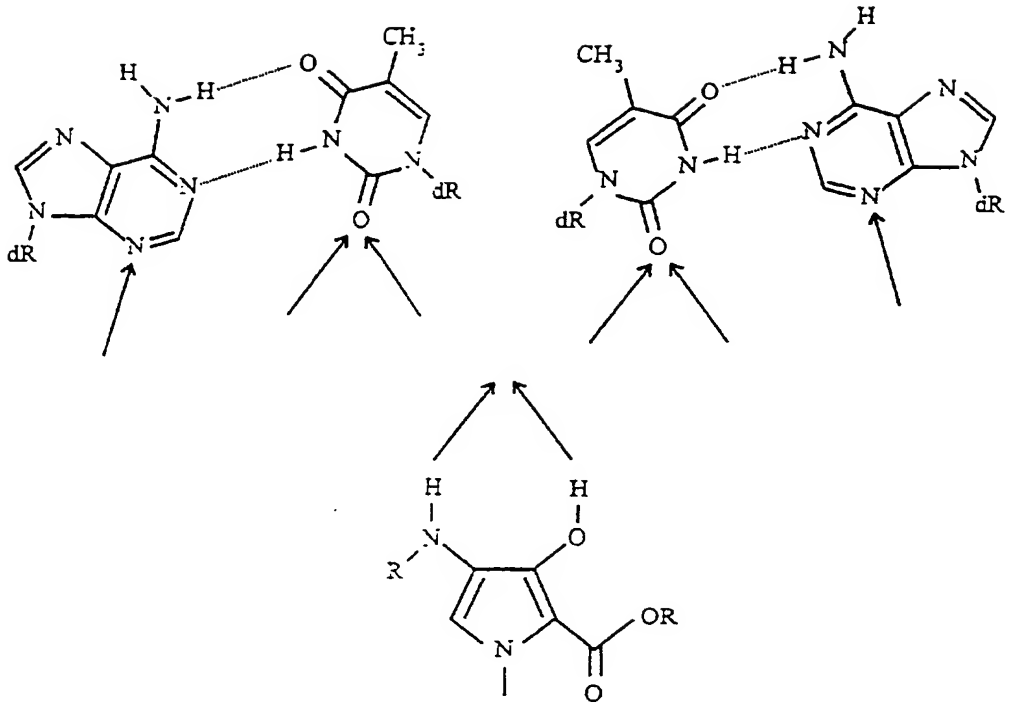


Fig. 5

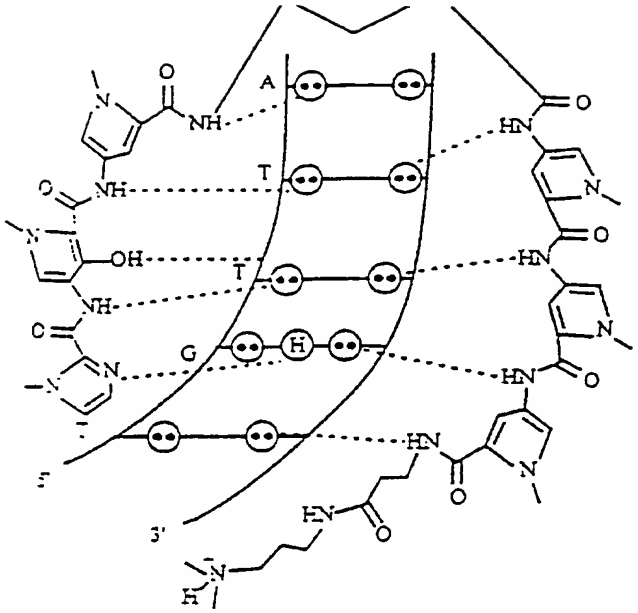


Fig. 6A

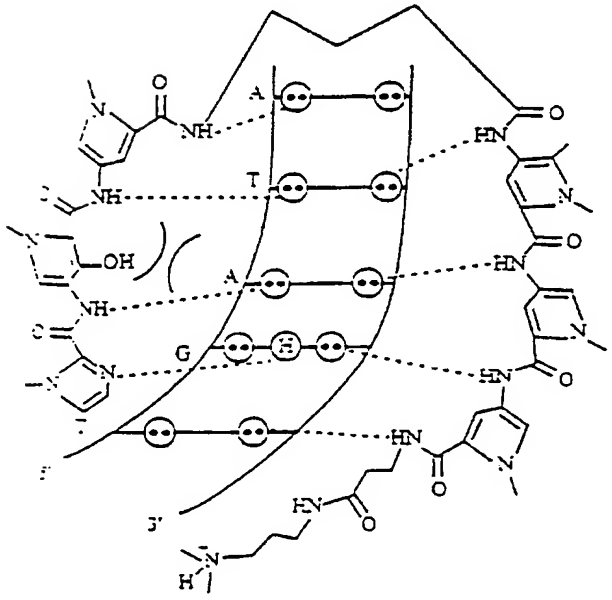
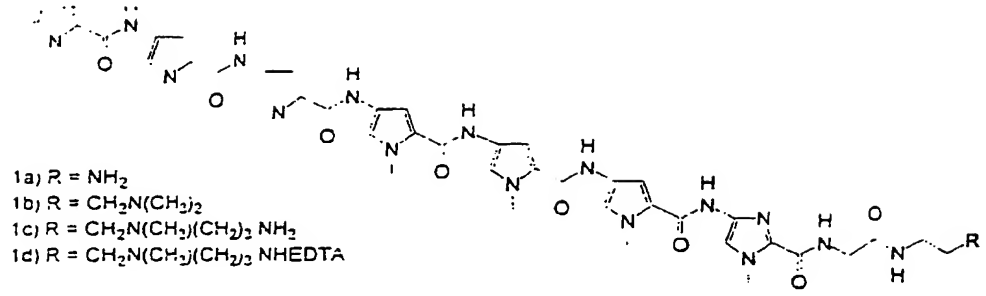
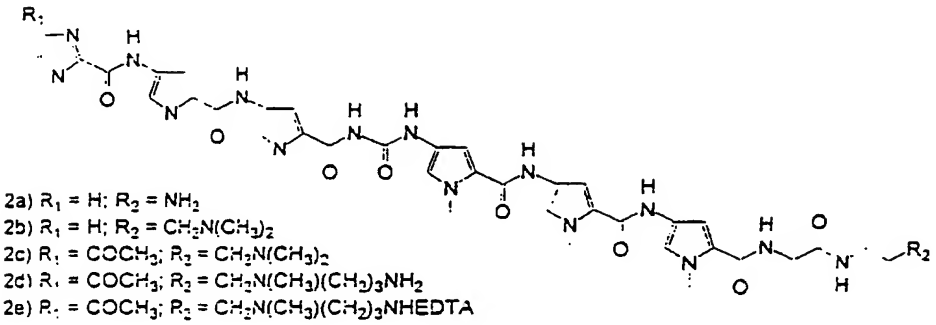


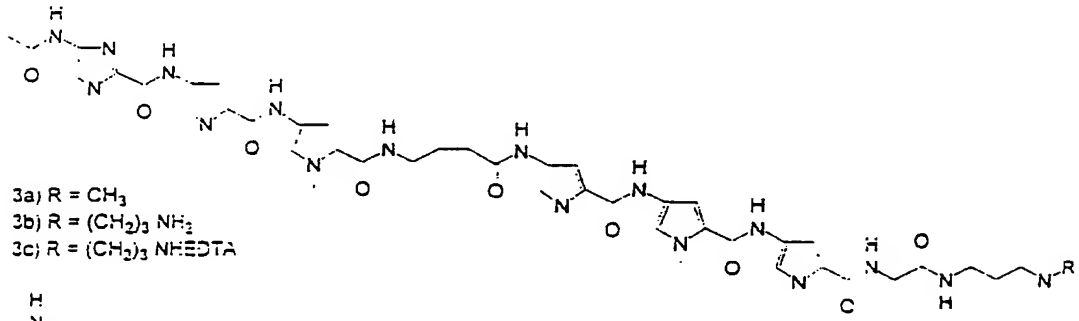
Fig. 6B



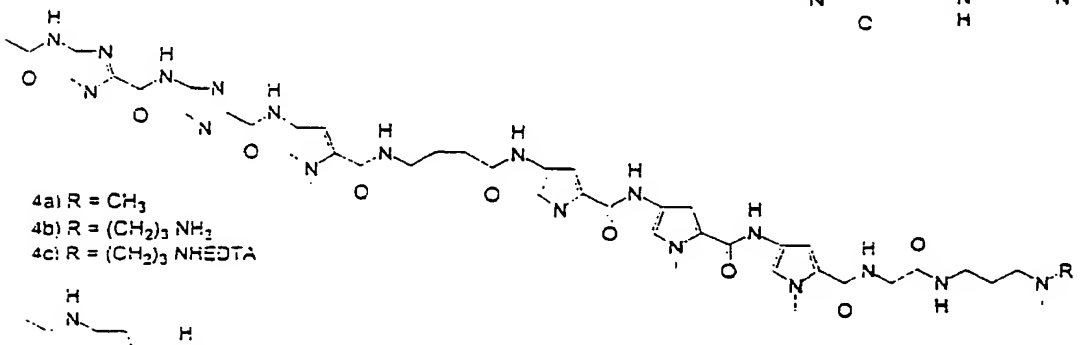
1a) R = NH_2
1b) R = $CH_2N(CH_3)_2$
1c) R = $CH_2N(CH_3)(CH_2)_2NH_2$
1d) R = $CH_2N(CH_3)(CH_2)_3$ NHEDTA



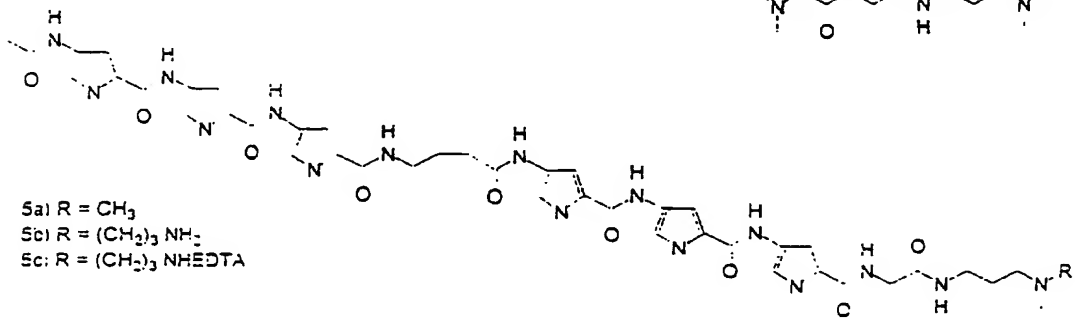
2a) R_1 = H; R_2 = NH_2
2b) R_1 = H; R_2 = $CH_2N(CH_3)_2$
2c) R_1 = $COCH_3$; R_2 = $CH_2N(CH_3)_2$
2d) R_1 = $COCH_3$; R_2 = $CH_2N(CH_3)(CH_2)_3NH_2$
2e) R_1 = $COCH_3$; R_2 = $CH_2N(CH_3)(CH_2)_3$NHEDTA



3a) R = CH_3
3b) R = $(CH_2)_3NH_2$
3c) R = $(CH_2)_3$ NHEDTA



4a) R = CH_3
4b) R = $(CH_2)_3NH_2$
4c) R = $(CH_2)_3$ NHEDTA



5a) R = CH_3
5b) R = $(CH_2)_3NH_2$
5c) R = $(CH_2)_3$ NHEDTA

Fig. 7

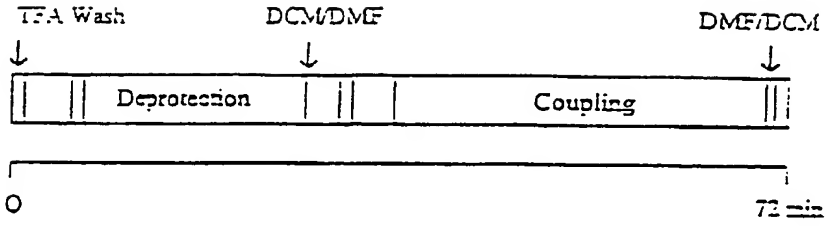


Fig. 8

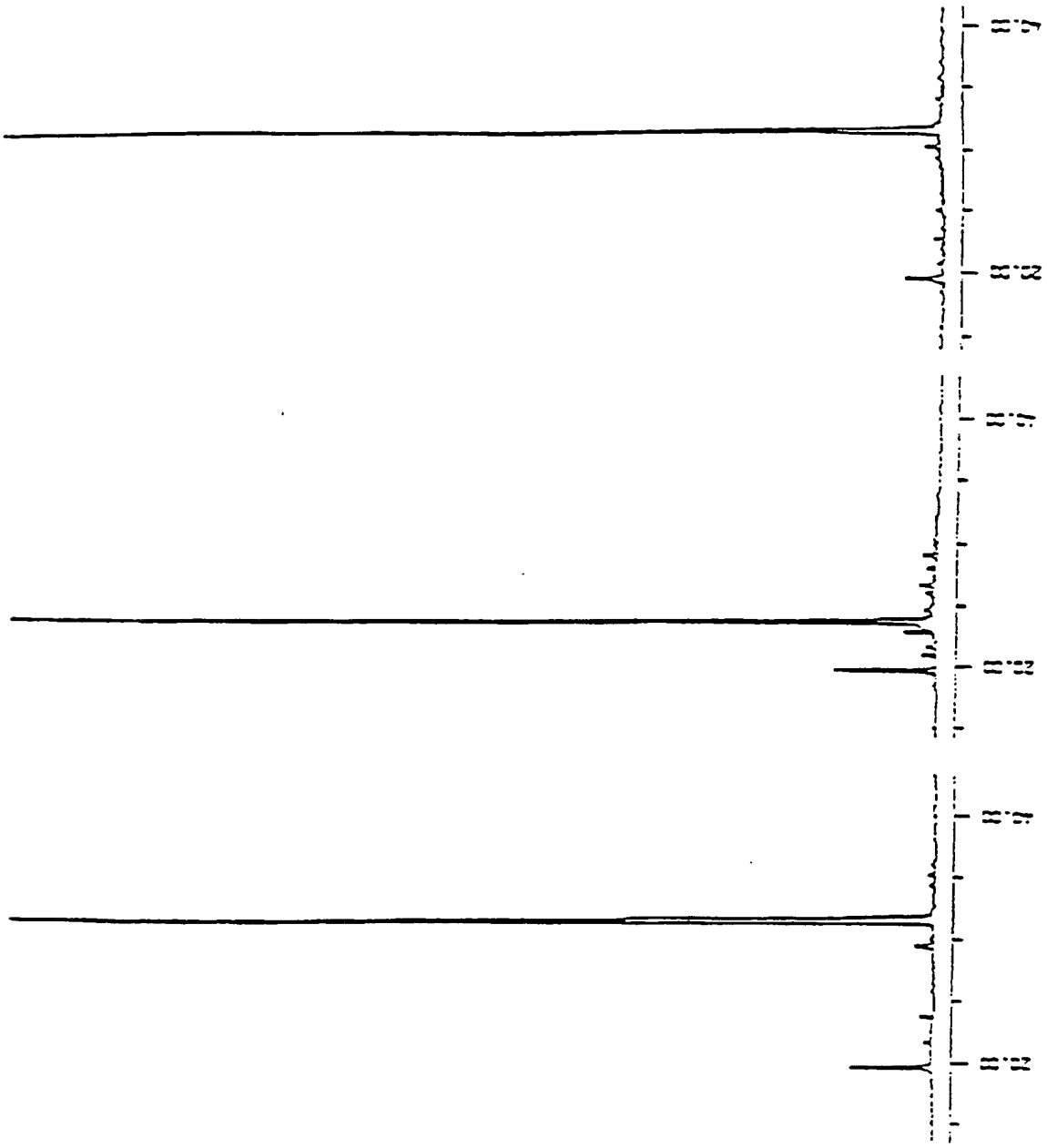


FIG. 9C

FIG. 9B

FIG. 9A

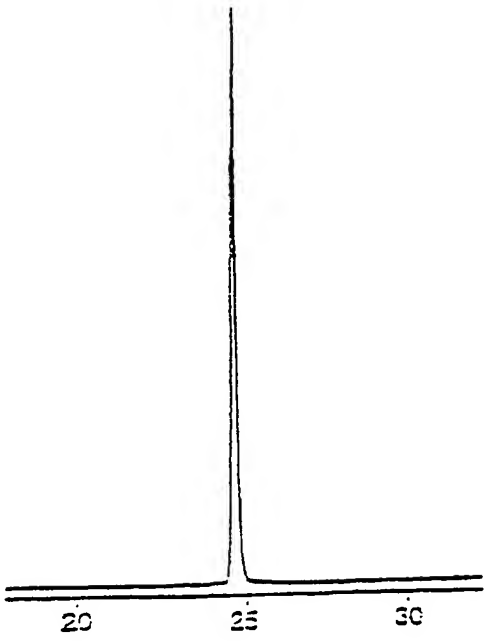


FIG. 10A

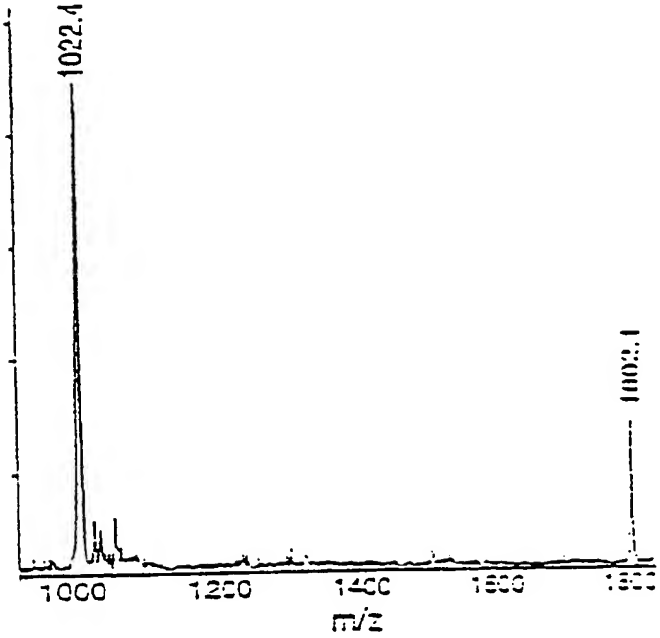


FIG. 10B

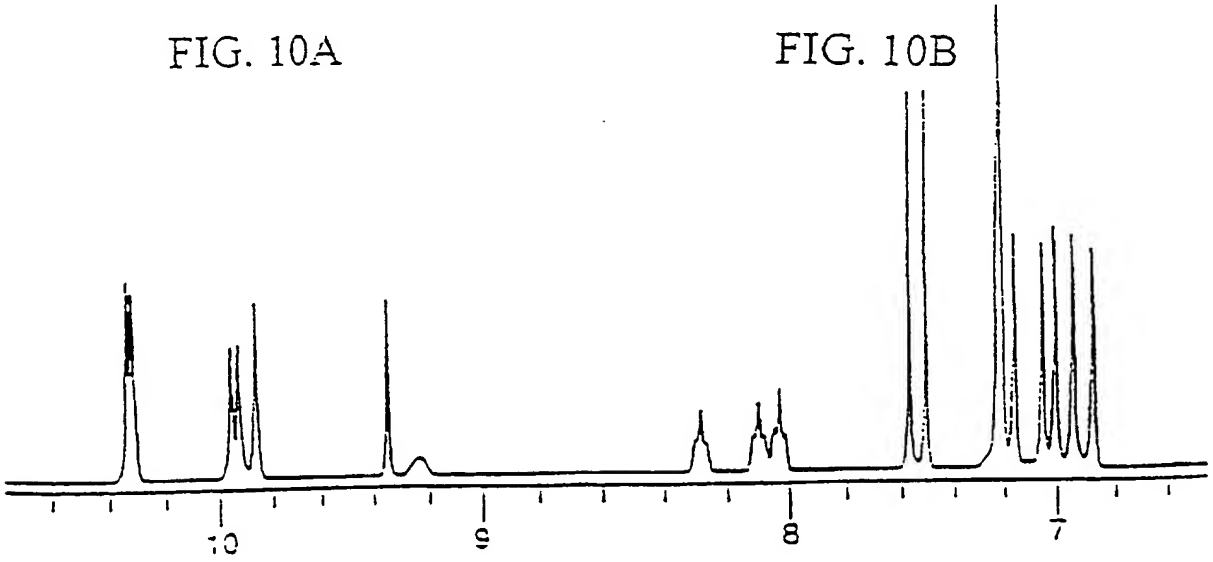
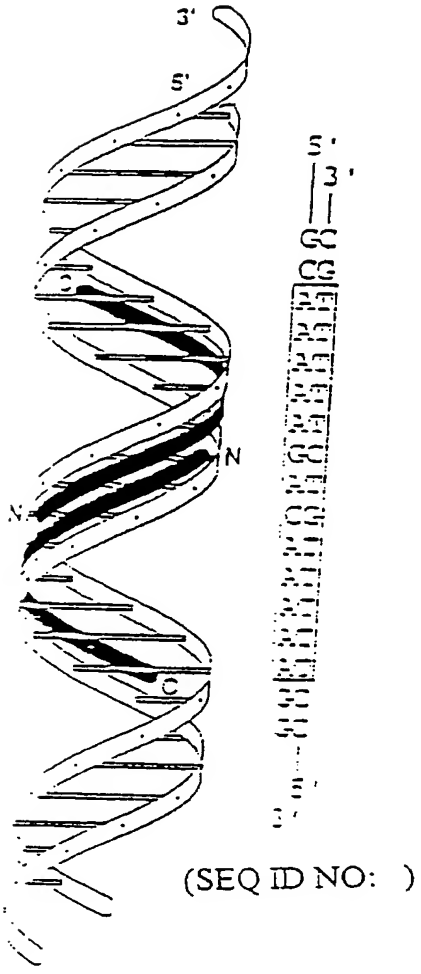
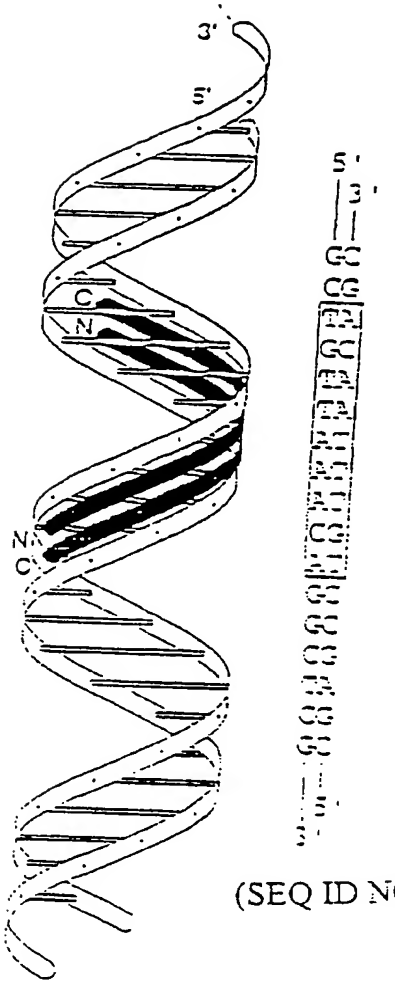


FIG. 10C



(SEQ ID NO:)

FIG. 11A



(SEQ ID NO:)

FIG. 11B

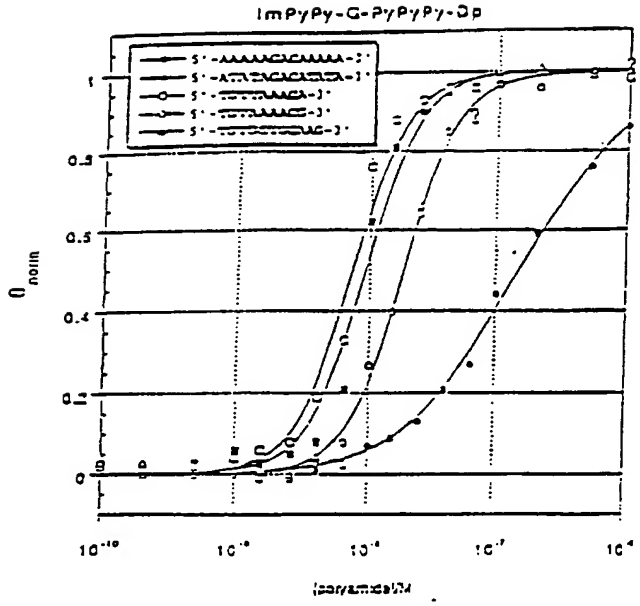


FIG. 12A

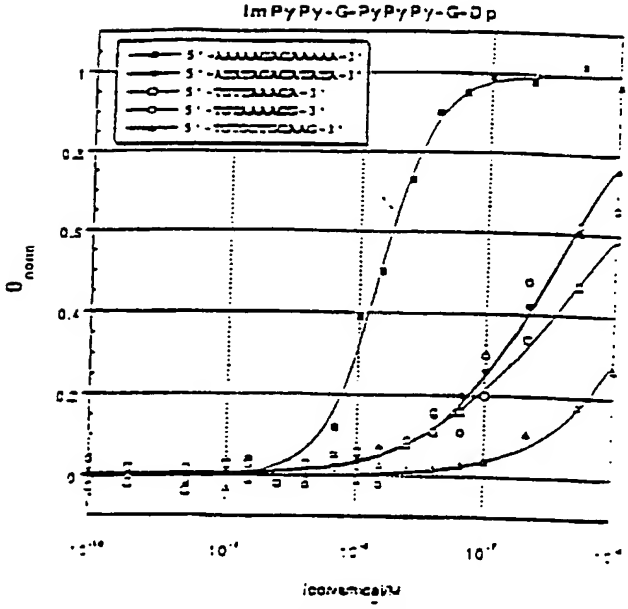


FIG. 12B

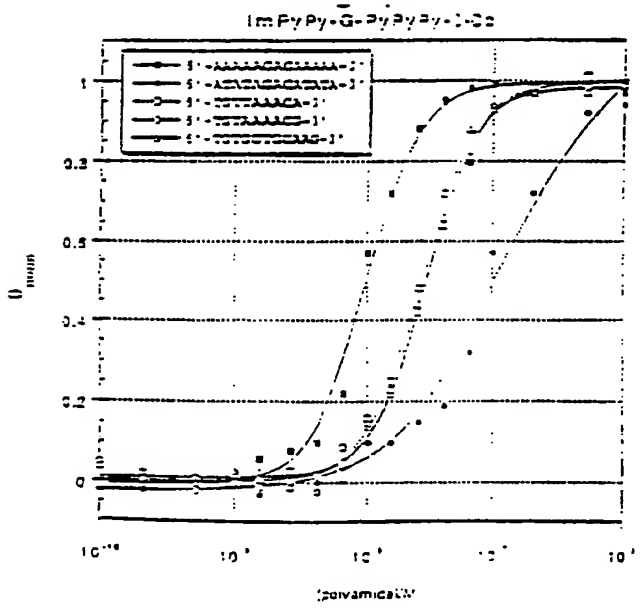


FIG. 12E

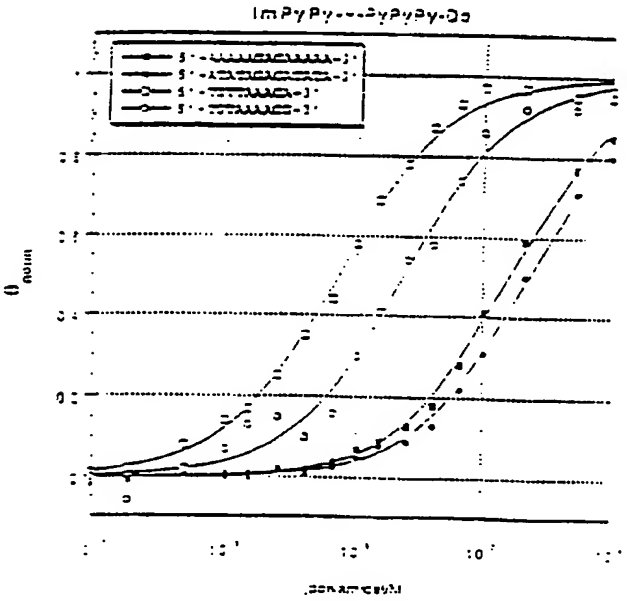


FIG. 12F

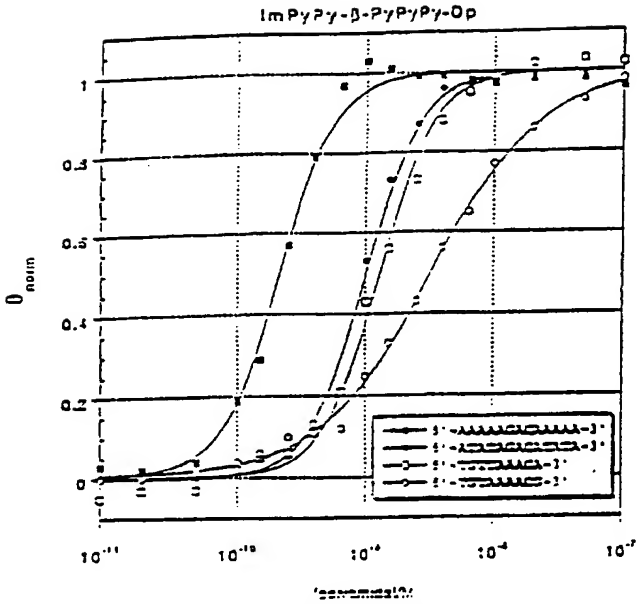


FIG. 12C

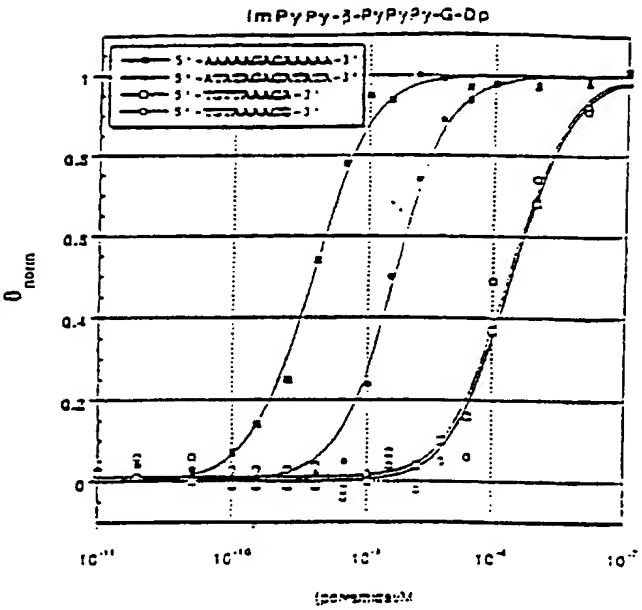


FIG. 12D

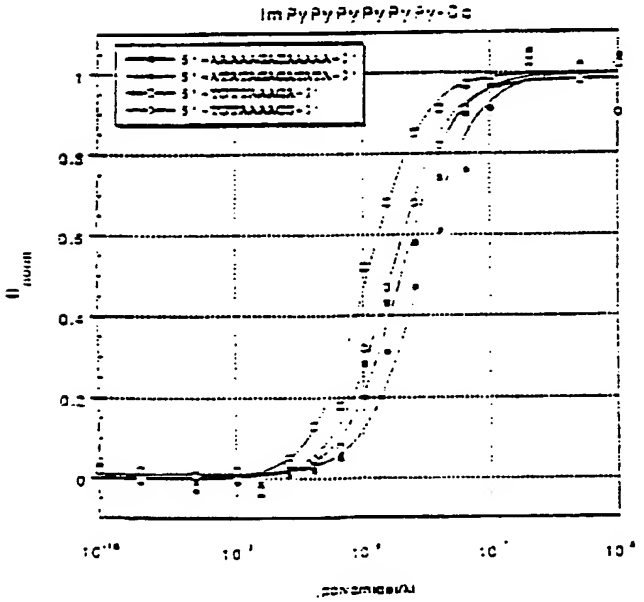


FIG. 12G

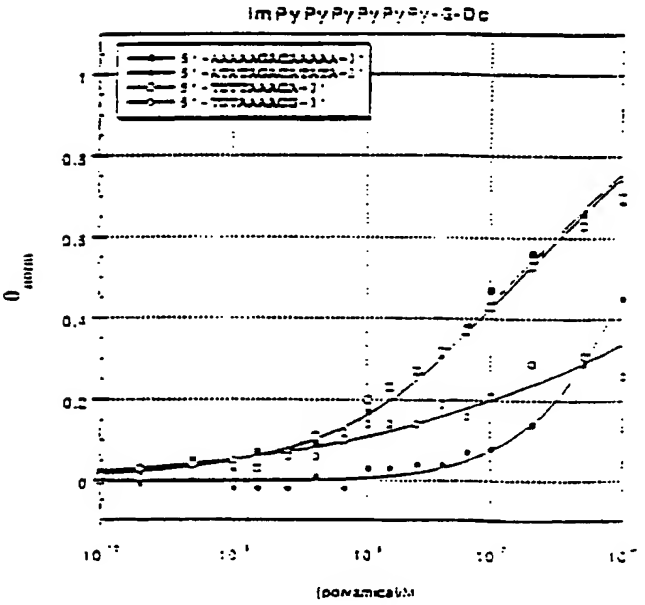


FIG. 12H

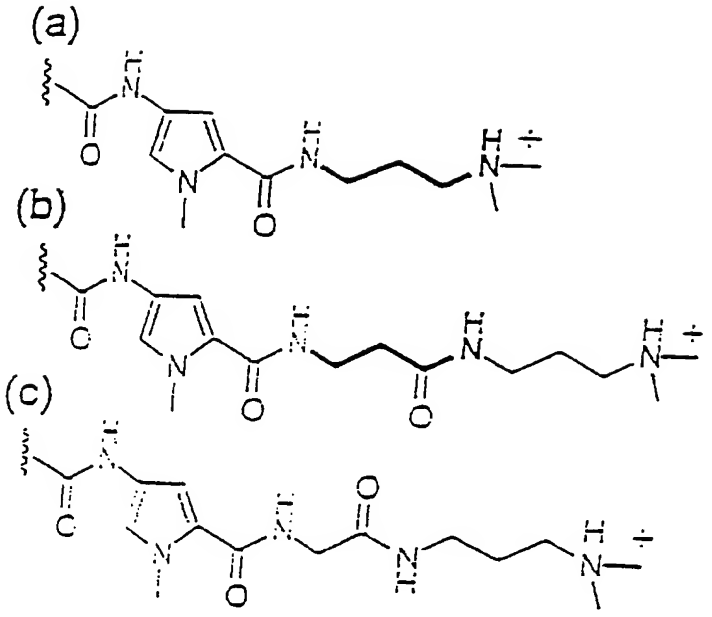


FIG. 13

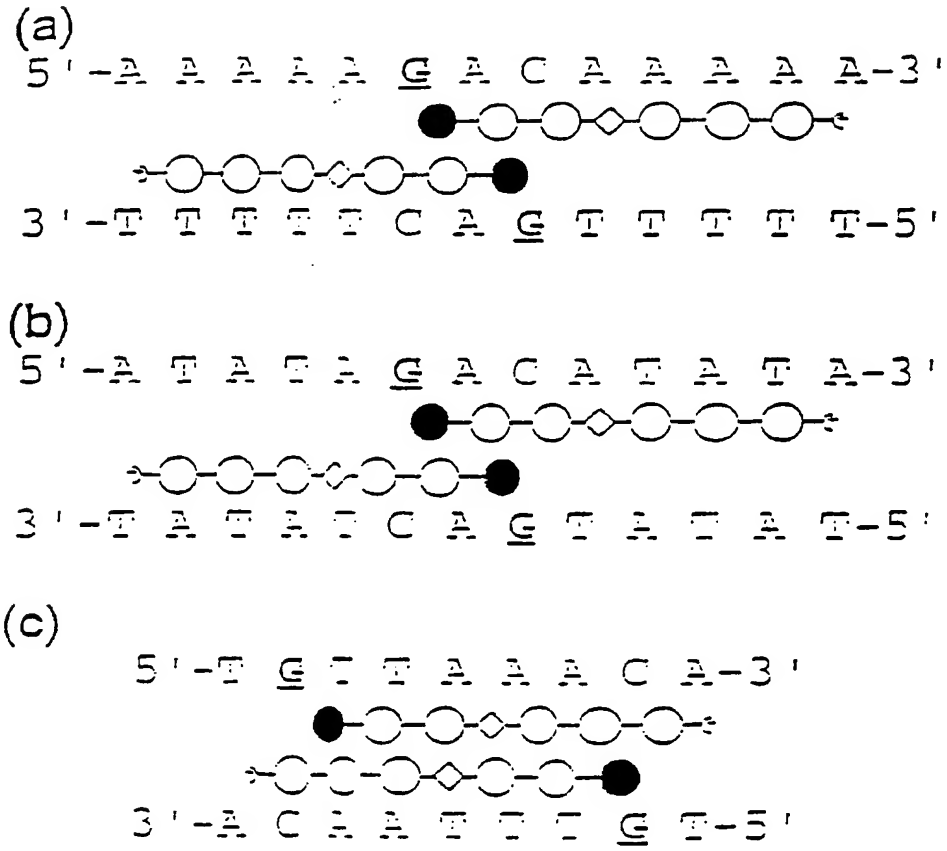

(a)
5'-A A A A A G A C A A A A A-3'
3'-T T T T T C A G T T T T T-5'
(b)
5'-A T A T A G A C A T A T A-3'
3'-T A T A T C A G T A T A T-5'
(c)
5'-T G T T A A A C A-3'
3'-A C A A T T T G T-5'

FIG. 14

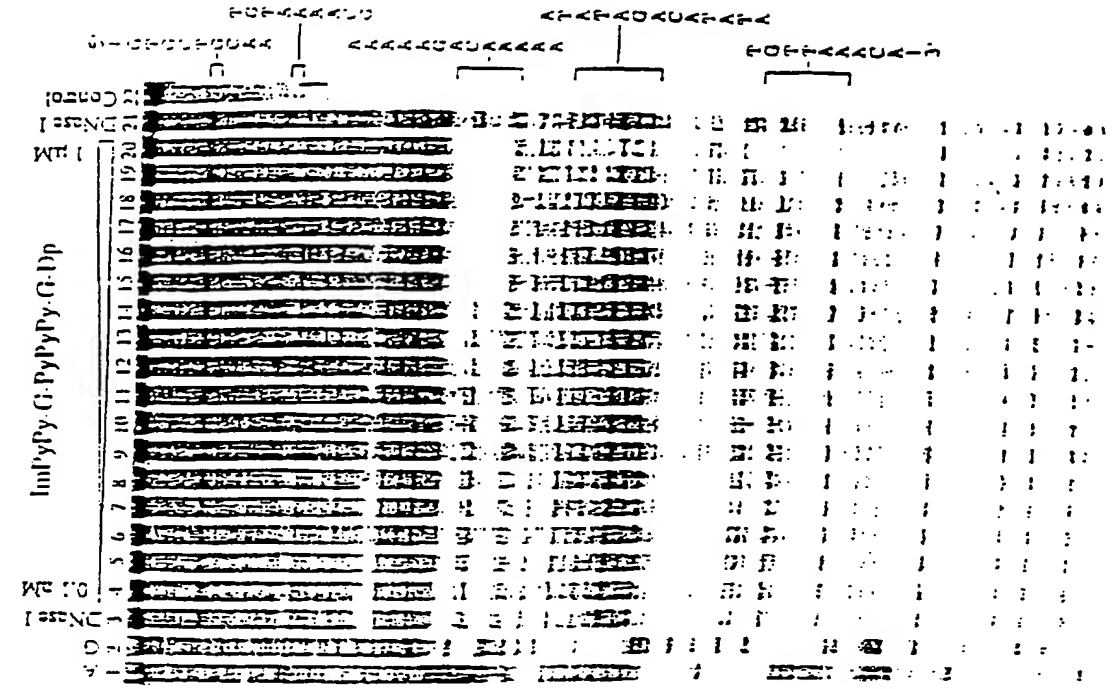


FIG. 15B

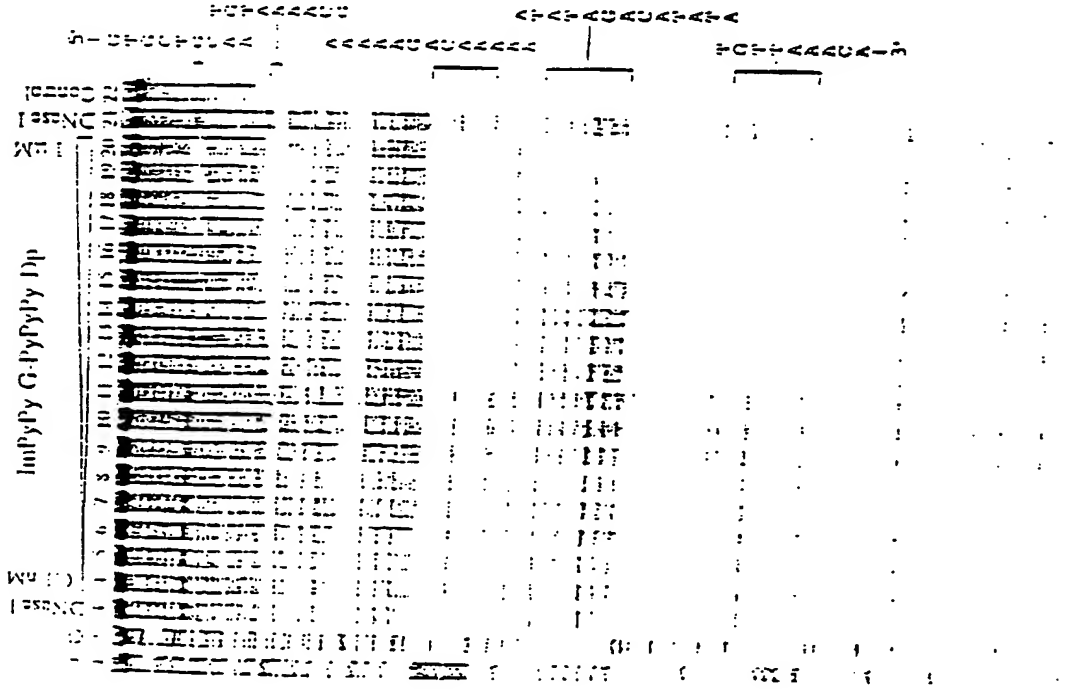


FIG. 15A

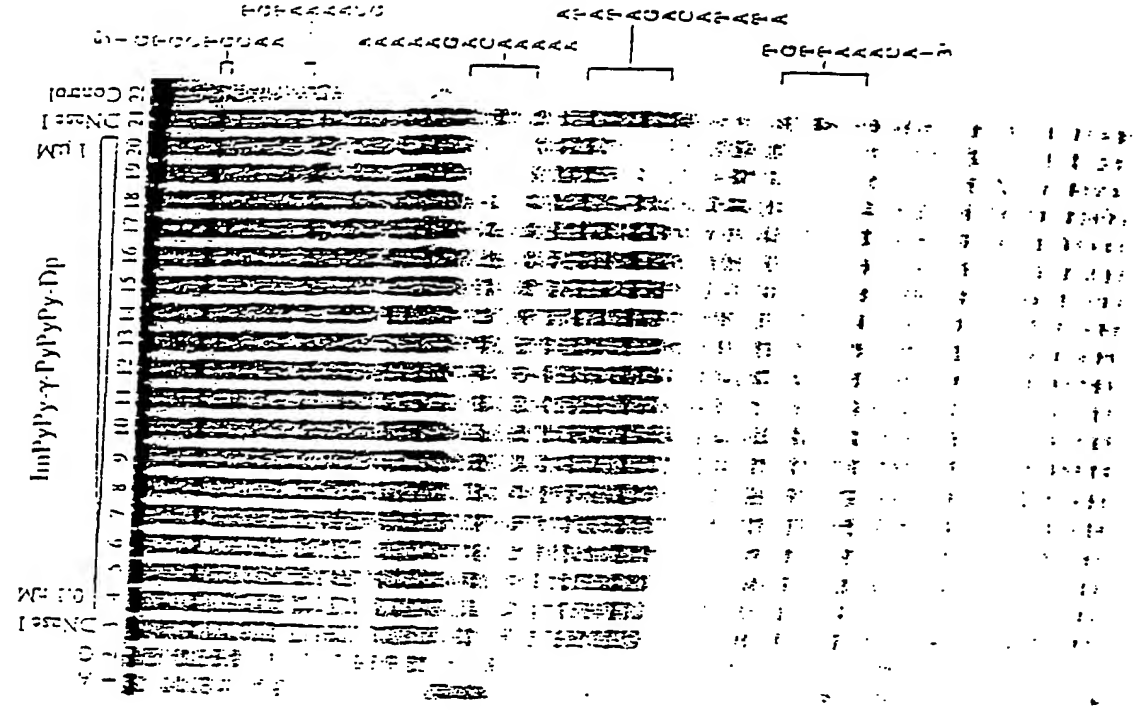


FIG. 15D

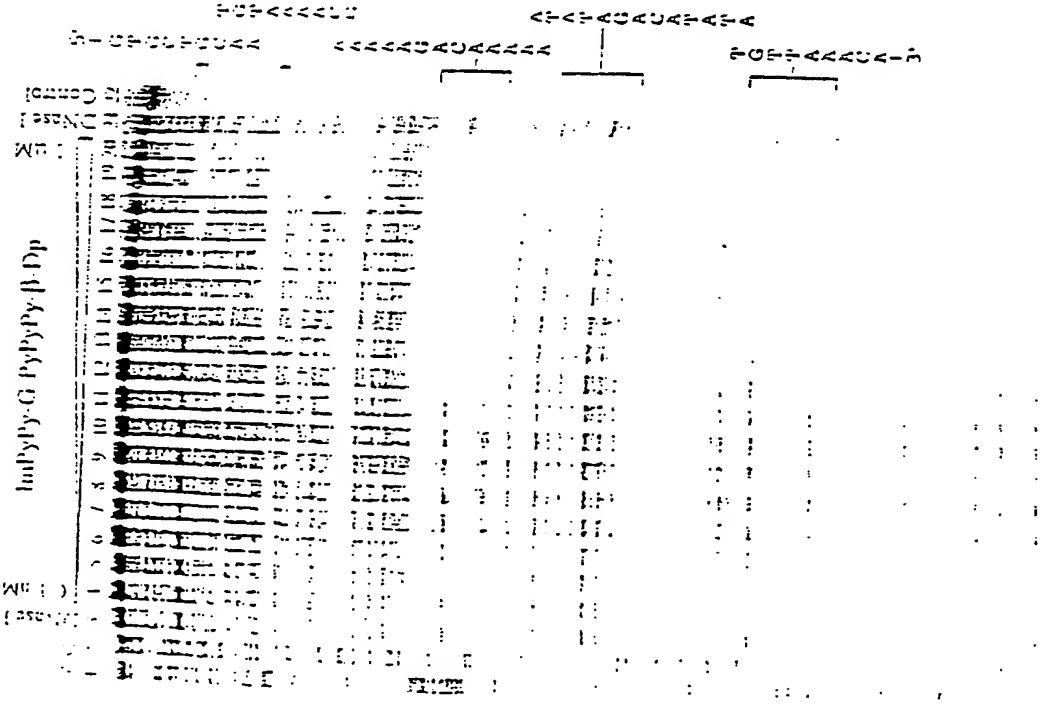


FIG. 15C

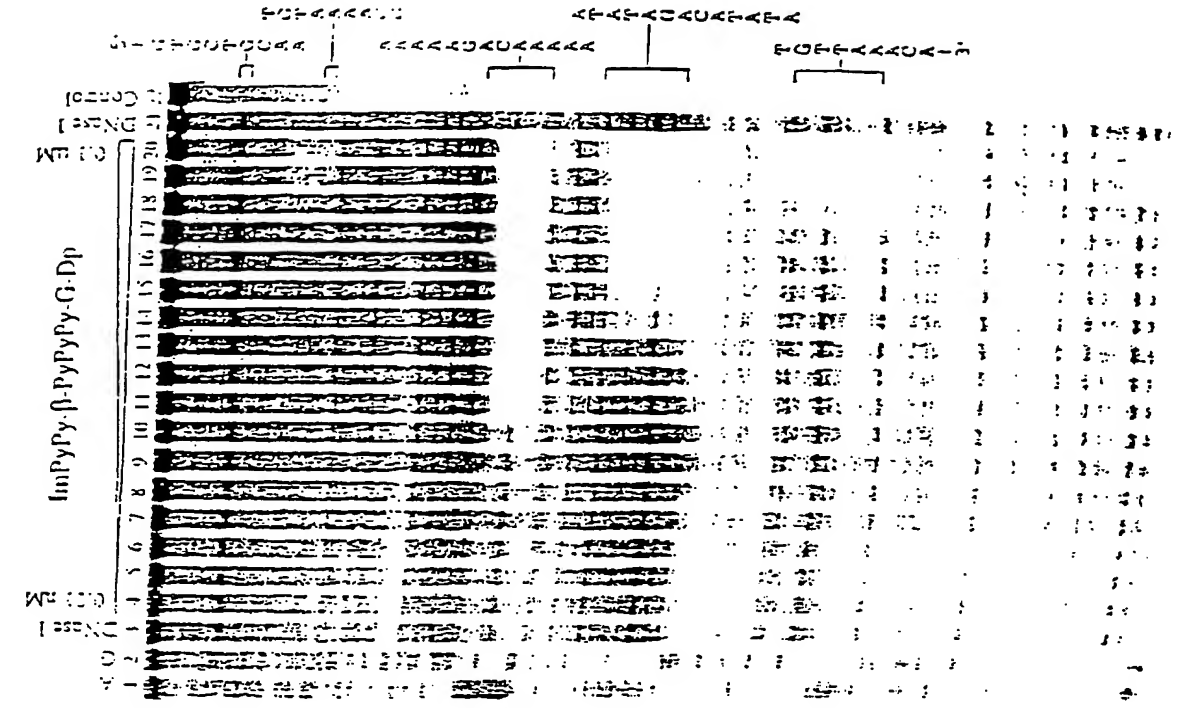


FIG. 15F

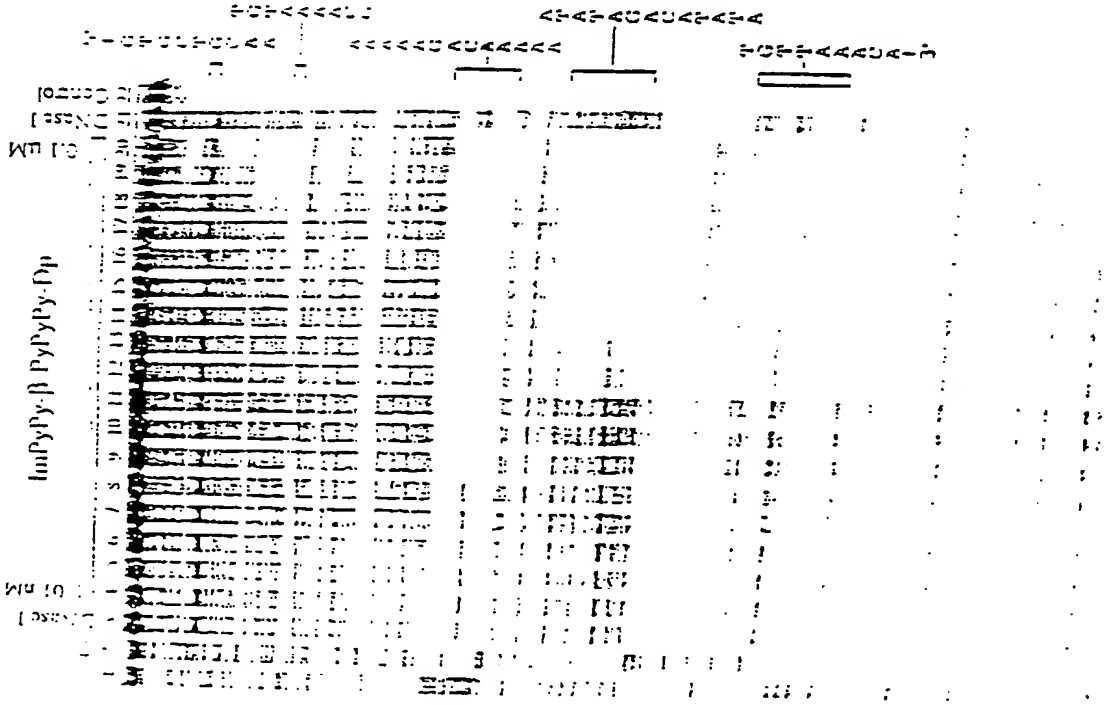


FIG. 15E

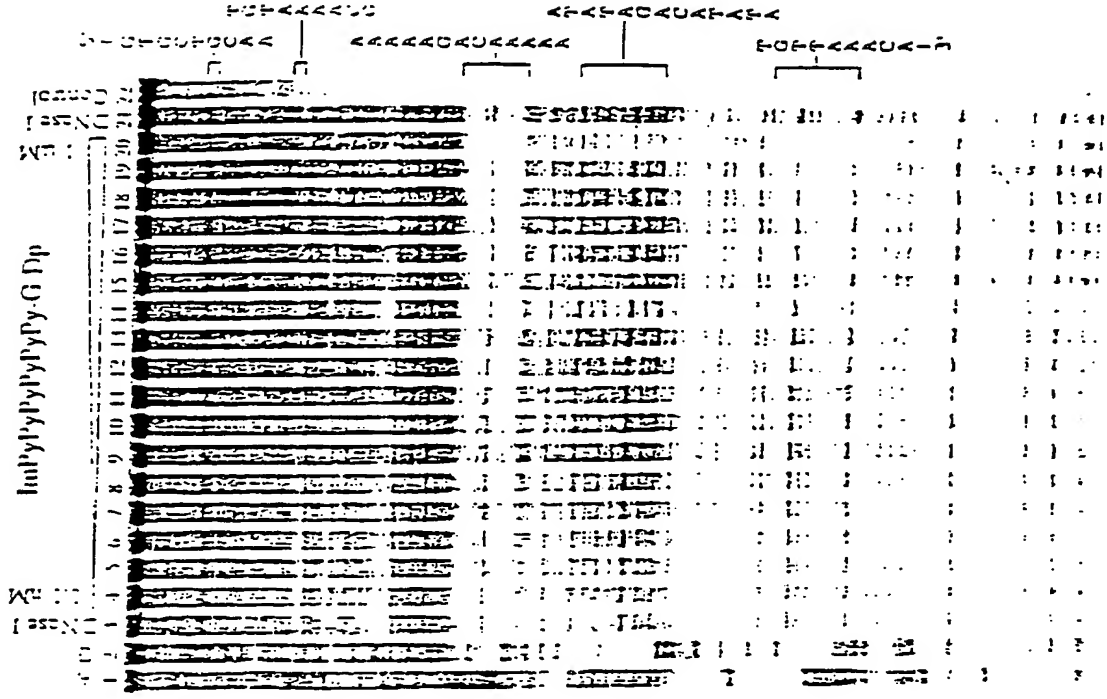


FIG. 15H

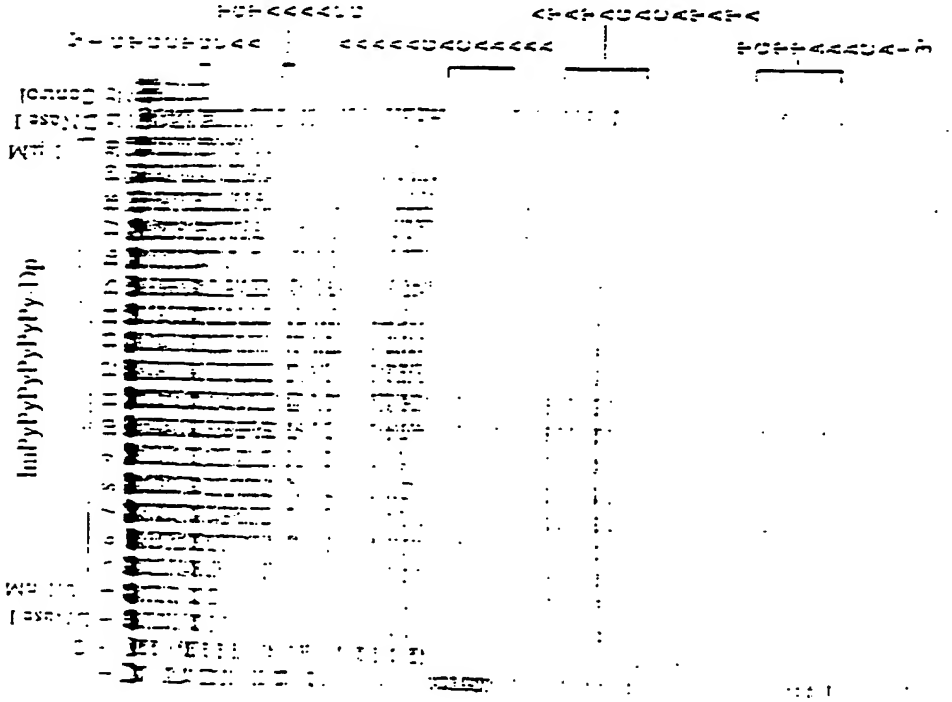


FIG. 15G

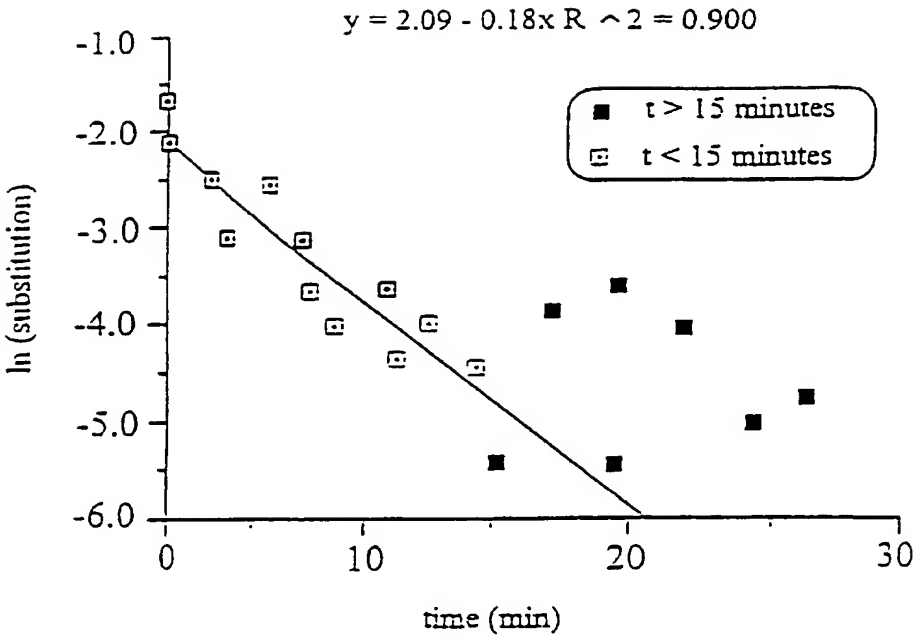

y = 2.09 - 0.18x R ^2 = 0.900
t > 15 minutes
t < 15 minutes
ln (substitution)
-1.0
-2.0
-3.0
-4.0
-5.0
-6.0
0
10
20
30
time (min)

Fig. 16

FIG. 17A

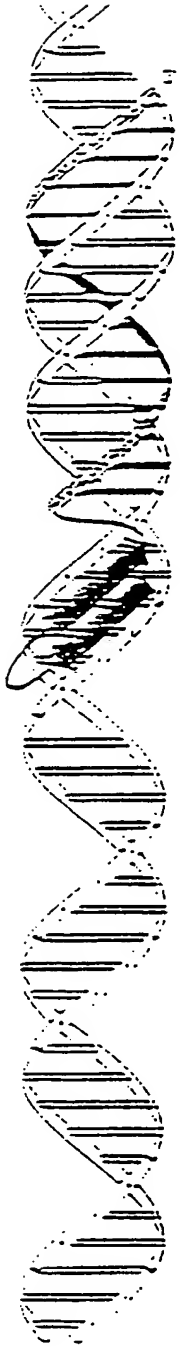
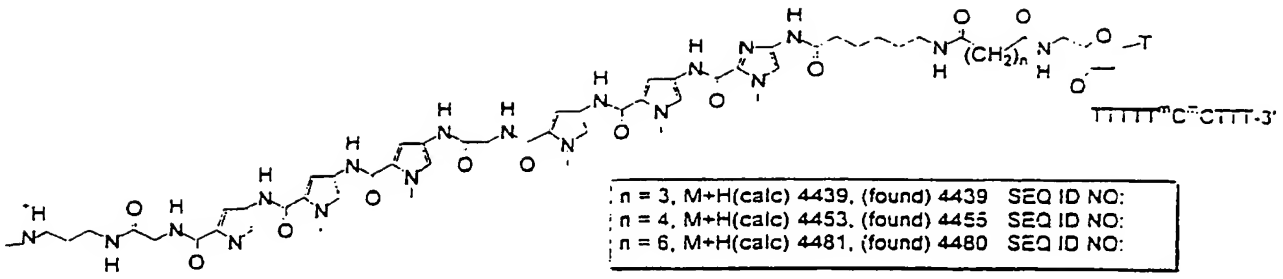
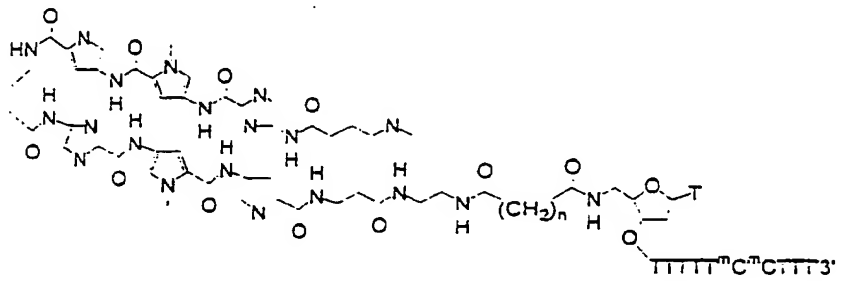


FIG. 17B



n = 3, M+H(calc) 4439, (found) 4439 SEQ ID NO:
n = 4, M+H(calc) 4453, (found) 4455 SEQ ID NO:
n = 6, M+H(calc) 4481, (found) 4480 SEQ ID NO:



n = 3, M+H(calc) 4444, (found) 4440 SEQ ID NO:
n = 6, M+H(calc) 4486, (found) 4487 SEQ ID NO:

Fig. 18

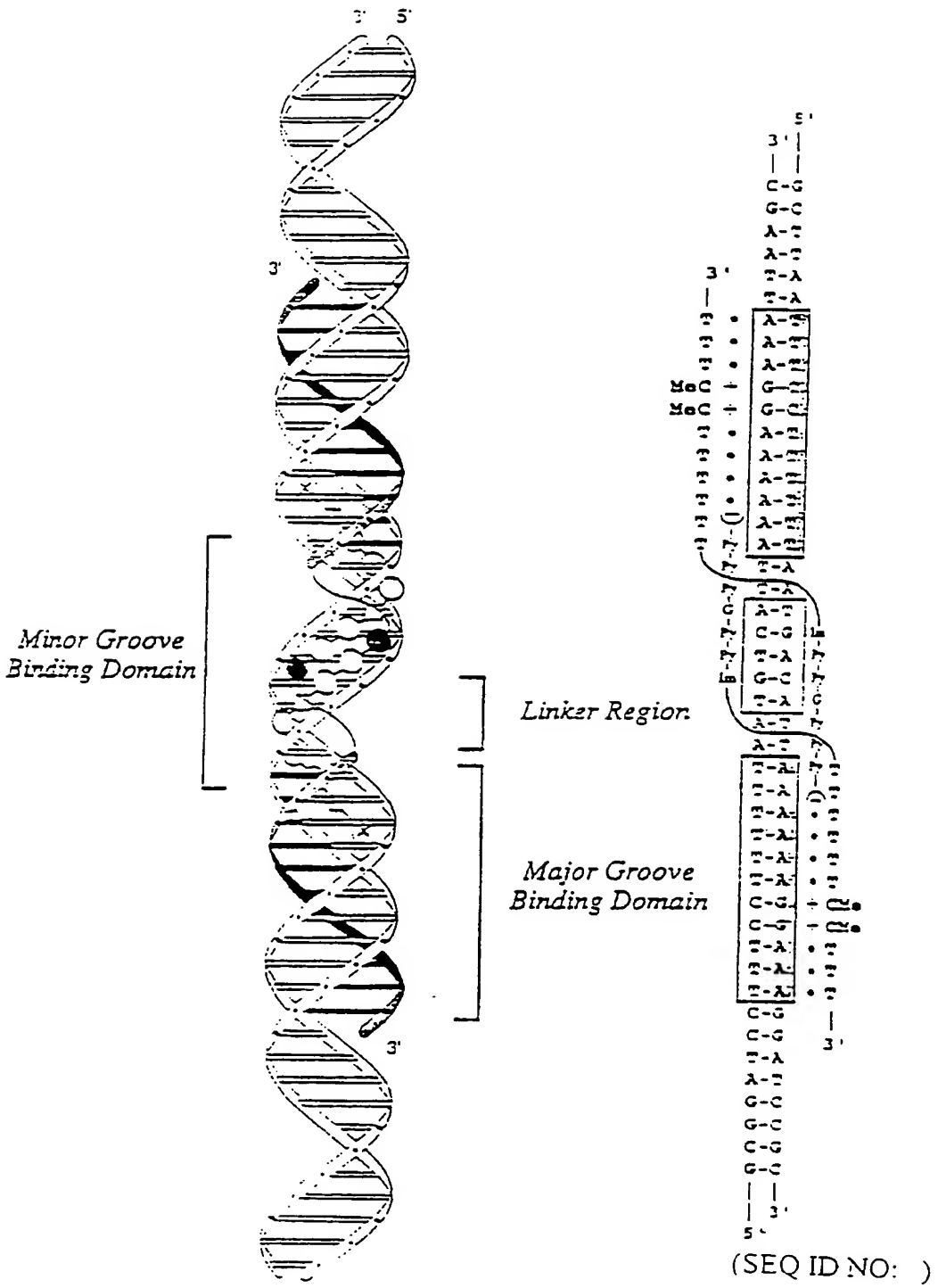

Minor Groove
Binding Domain
Linker Region
Major Groove
Binding Domain
(SEQ ID NO:)

FIG. 19

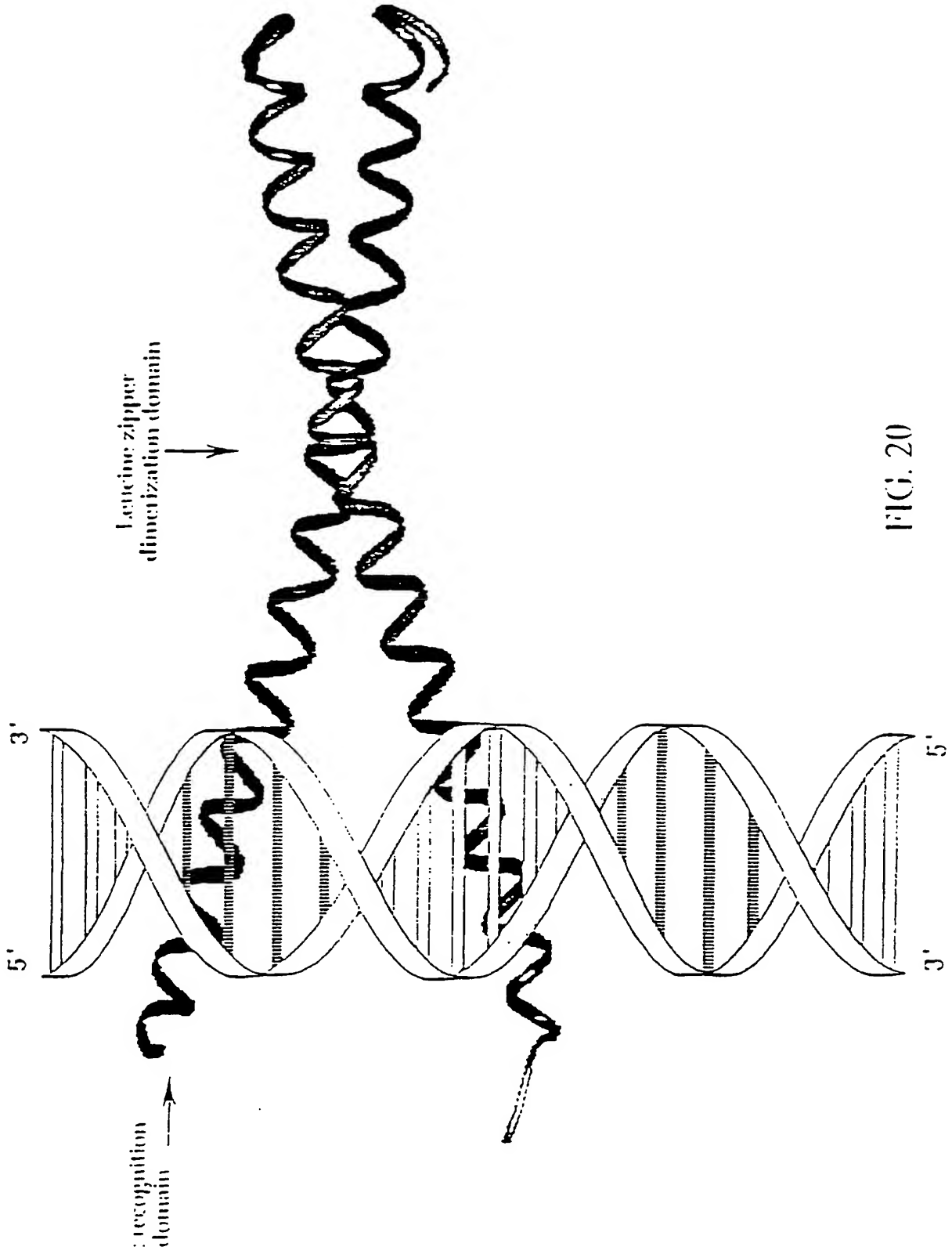

Leucine zipper dimerization domain
3'
5'
5'
3'
recognition domain

FIG. 20

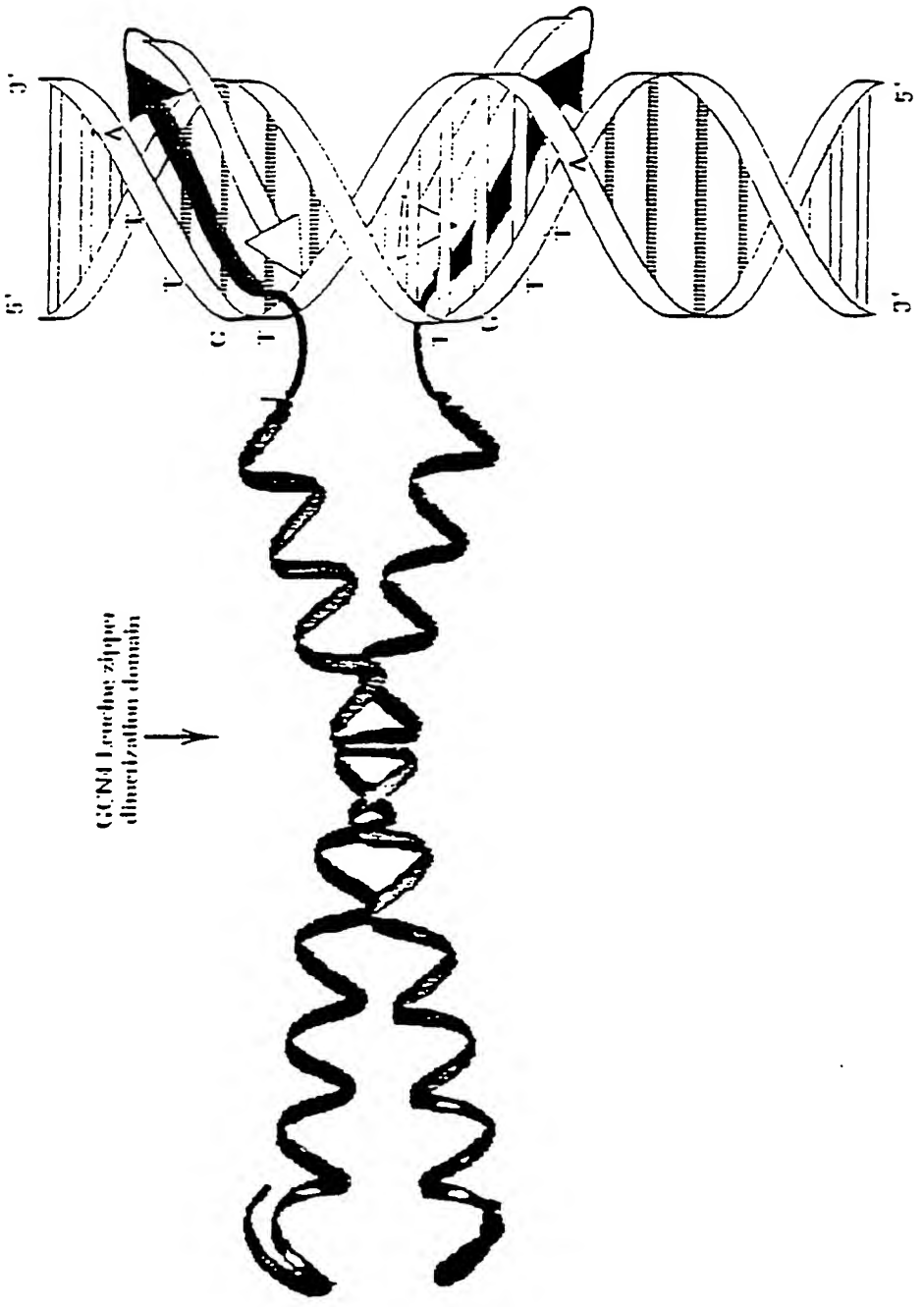


FIG. 21

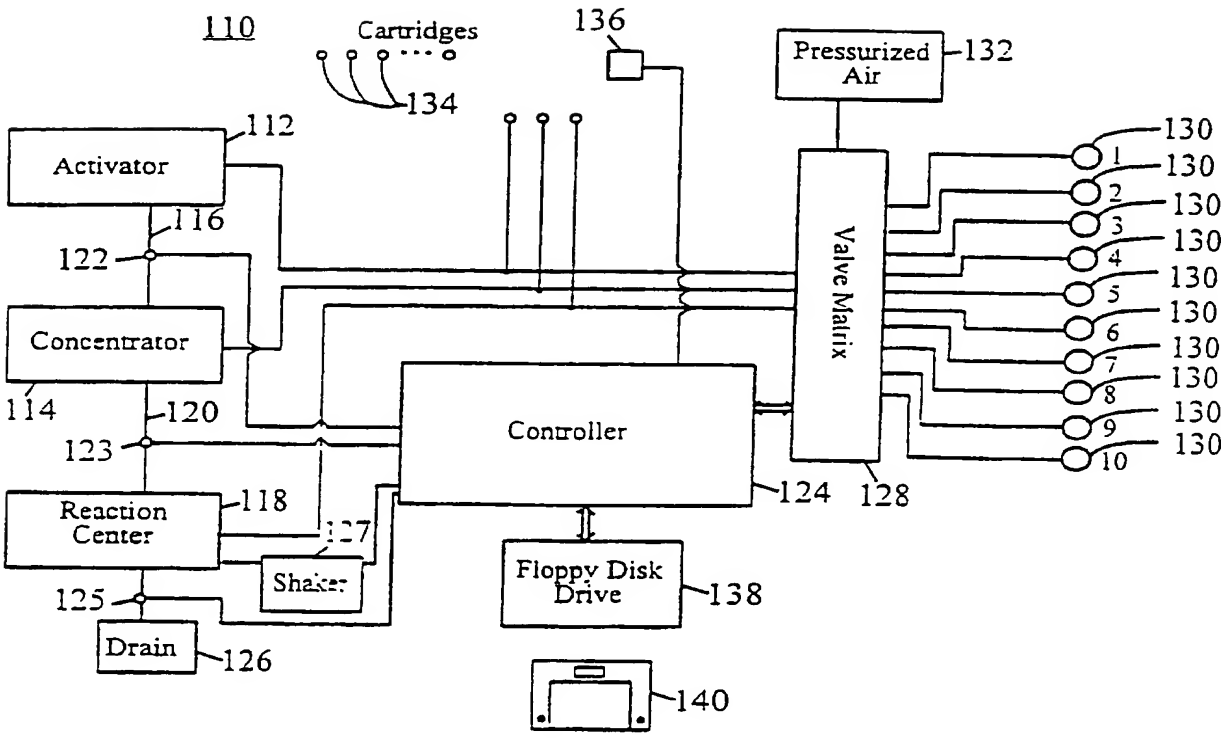

110
Cartridges
134
136
Pressurized Air
132
112
Activator
116
122
Concentrator
114
120
123
Reaction Center
118
127
Shaker
125
Drain
126
Controller
124
128
Valve Matrix
130
1
2
3
4
5
6
7
8
9
10
Floppy Disk Drive
138
140

Fig. 22

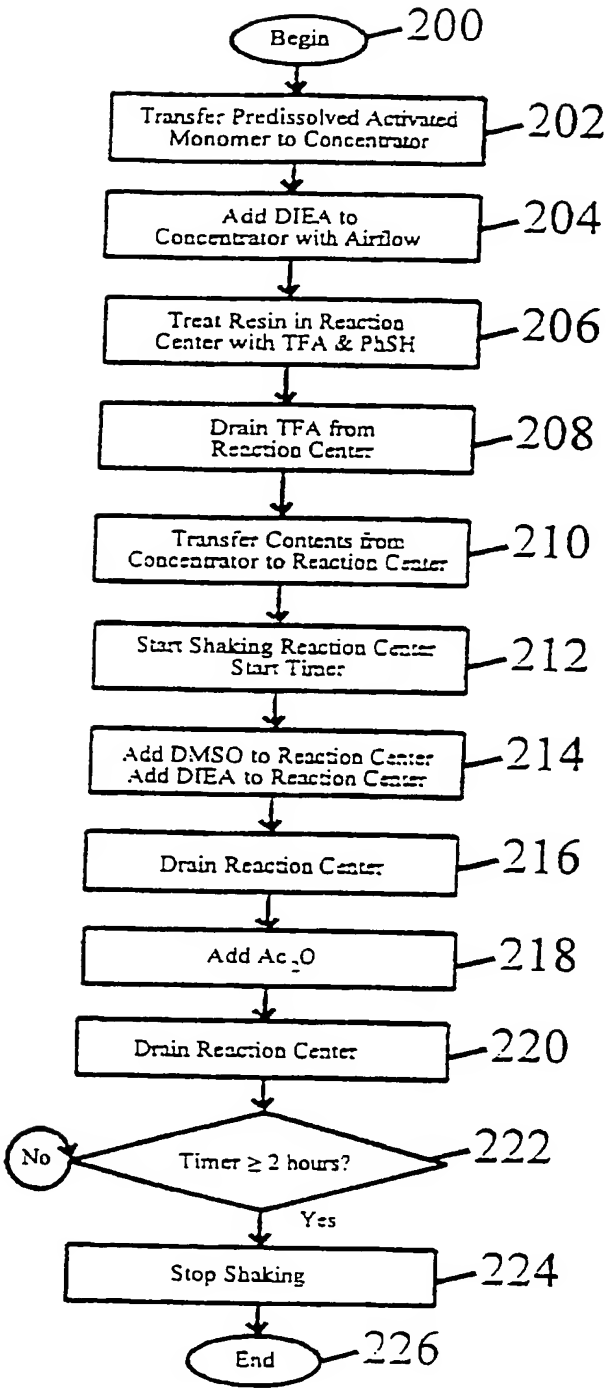

Begin
200
Transfer Predissolved Activated Monomer to Concentrator
202
Add DIEA to Concentrator with Airflow
204
Treat Resin in Reaction Center with TFA & PhSH
206
Drain TFA from Reaction Center
208
Transfer Contents from Concentrator to Reaction Center
210
Start Shaking Reaction Center Start Timer
212
Add DMSO to Reaction Center Add DIEA to Reaction Center
214
Drain Reaction Center
216
Add Ac2O
218
Drain Reaction Center
220
No
Timer ≥ 2 hours?
222
Yes
Stop Shaking
224
End
226

Fig. 23

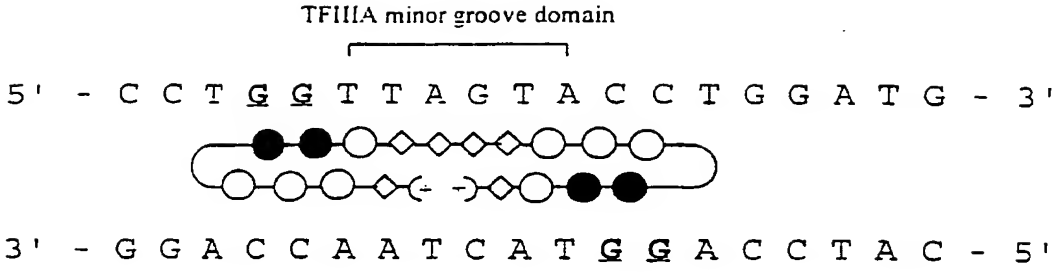

TFIIIA minor groove domain
5' - C C T G G T T A G T A C C T G G A T G - 3'
3' - G G A C C A A T C A T G G A C C T A C - 5'

Fig. 24